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SYMBIOTIC NITROGEN FIXATION AS INFLUENCED BY THE NITROGEN IN THE SOIL[1]

WILLIAM ALBERT ALBRECHT

Formerly Fellow in Agronomy, University of Illinois[2]

Received for publication February 10, 1920

I. INTRODUCTION

Of the important elements necessary for growing plants, nitrogen is the one that presents our most serious problem and is most apt to be deficient. Early studies on the different forms in which nitrogen may serve for plant growth seemed to have it definitely settled that only combined nitrogen could be used by plants (6, 30). Atwater (2), however, showed that legumes, quite contrary to earlier beliefs, were able indirectly to utilize the elementary nitrogen. This prompted many researches and opened many discussions, which soon established the fact that certain plants, belonging to the *Leguminosae*, were able to obtain the gaseous nitrogen of the air through the action of bacteria living in the nodules on their roots.

When the fact became known that legumes are able to use the nitrogen of the air through a mutually beneficial relationship with bacteria, numerous studies of these plants were undertaken to determine the manner in which they take nitrogen from the air and incorporate or fix it in their tissues.

This process of "symbiotic nitrogen fixation," as it has been named, has taken on considerable significance in the attempt to maintain the nitrogen supply for plant growth. The fact that it offers a means of utilizing the unlimited supply of nitrogen of the air in place of the costly nitrogenous fertilizers, has served as an incentive to study this process and the factors which influence its highest development. Any information giving a clearer understanding of the process of symbiotic nitrogen fixation may be justified as contributing to the large agricultural problem of maintaining the supply of nitrogen in the soil in sufficient amounts to insure maximum crop production. The following research is a contribution to the process of nitrogen fixation by legumes as influenced by the amount of nitrogen present in the soil in both the organic and inorganic forms.

[1] A thesis submitted to the Faculty of the Graduate School of the University of Illinois in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, June, 1919.

[2] Present Associate Professor of Soils, University of Missouri.

II. HISTORICAL

The literature on the subject of symbiotic nitrogen fixation is rather extensive and has been well collected in bibliographies by Jacobitz (27), by Burrill (7), and others, so that no extensive review on the subject is necessary. Only those papers dealing particularly with this process as influenced by the nitrogen content of the soil will be considered.

General statements are common in saying that the legume fulfills its needs for nitrogen from the soil and later resorts to the supply in the air. Conn (8) makes the statement that "legumes appear to prefer taking their nitrogenous material directly from the nitrogenous foods in the soil when these are present in abundance. But if the soil does not furnish the proper nitrogen, then recourse is had to atmospheric nitrogen, through the agency of tubercle organisms." Van Slyke (46) ventures a similar opinion in which he says, "when supplied with available nitrogen compounds, the bacteria fail to make use of atmospheric nitrogen." Hopkins (25, p. 217) agrees in substance with this. "Clover and other legumes," he says, "take available nitrogen from the soil in preference to the fixation of free nitrogen from the air, the latter being drawn upon only to supplement the soil's supply and thus balance the plant-food ration."

Early works show that nodules are present when the bacteria become established and when the plant uses atmospheric nitrogen. In much of the literature the nodules represent nitrogen fixation and unless this modification of the root is present, no use of gaseous nitrogen is believed to be taking place. The importance of nodules in nitrogen fixation was established early by Hellriegel and Wilfarth (19) as one of the fundamental facts when they say, "the nodules of the roots must not be considered as simply reservoirs of albuminoid substances; their relation to the assimilation of free nitrogen is that of cause to effect." In most works cited in the following discussion, the effects of the nitrogen in the soil as a cultural medium, are reported as favorable or unfavorable to the nodule production, and hence to the nitrogen fixation.

Moore (34) apparently does not agree with this general conception of the importance of nodules. He believes that it is possible for the bacteria to enter the roots and be of benefit without evincing their presence by such external evidence as nodules. He fails to believe that even if the absence of nodules might permit some nitrogen fixation, this would not prohibit the same performance in their presence.

Rautenberg and Kuhn (43) ventured perhaps the earliest statement concerning the relation of nitrogen fixation to the nitrogen in the medium for plant growth. In their work *Vicia faba*, growing in a nitrogen-free solution, developed numerous nodules, while in the presence of nitrates no nodules appeared. De Vries (10) obtained similar results while studying nodules as a storage for nitrogen. In the absence of nitrogen from the cultural solution, many nodules of normal structure were produced, but otherwise scarcely any developed.

Schindler (44) working with clovers, vetch, serradella, kidney vetch, and beans in water cultures concludes that, in general, solutions rich in nitrogen are less disposed toward giving nodules than those lacking in this element. These same plants grown in soil fertilized with compost or manure as compared with soils low in nitrogen gave larger and more numerous nodules in every case with the latter soil. Tschirch (45) in studying nodules, as a means of nitrogen storage, says that it is established that nodules grow more profusely in soils poor in nitrogen than in those rich in humus.

Vines (47) treated soils with 1 per cent potassium nitrate and found nodular development decreased, and also an indication that as the amount of nitrate diminished, the development of nodules became more marked. Similar depressive effects on nodule production by nitrates are reported also by Baszler (3) and by Laurent (28).

These studies on the influence of the nitrogen content of the soil on the nodule development, take this development as a measure of nitrogen fixation, but fail, however, to substantiate their contentions with careful chemical analyses to show that the total nitrogen in the plant and soil has truly increased.

Frank (13) studied conditions of the plant as influenced by inoculation and other factors. In using soils rich in humus as compared with those very poor in this respect, he was led to believe that when humus is present in sufficient amounts the bacteria are dispensable and serve with no benefit to the plant. Where humus is lacking the bacteria are active. This explains why legumes can be grown on sand when all minerals are supplied even when no humus is present.

Atwater (2) in growing peas in sand supplied with nutrient solutions containing varying amounts of potassium and calcium nitrates, found nitrogen fixation taking place. He measured it by the increase in the total nitrogen present at the close of the experiment as compared with that at the start. Table 1 is taken from his data and shows fixation when a large nitrogen ration is supplied.

Woods (50) (of Connecticut) grew scarlet clover in sand with a nutritive solution and accounted for all the nitrogen in the experiment, as did Atwater, by analysis of material at the outset and at the close. With no nitrogen in the solution, 18 plants fixed an average of 37 mgm. per plant. When 40 mgm. of nitrogen as calcium nitrate and potassium nitrate were added at the start, the fixation was reduced to 30 mgm. per plant. Vetch responded differently, fixing 20 mgm. in the former case and 47 in the latter. Cowpeas treated with nitrate fixed amounts varying from 87 to 129 mgm. of nitrogen. His data show a larger part of the nitrogen in the roots when nitrate was omitted than when it was added. He says, "all the plants grown without added nitrogen gained in nitrogen. Some of the plants supplied with nitrate showed a loss. The gain has occurred where root nodules are developed and without them there was no gain of any account."

Prazmowski (42) worked on an experiment similar to that of Woods to find out if the nitrates in the soil hindered or aided the bacteria in entering the

plant and fixing nitrogen. Peas in sterile sand cultures containing 300 mgm. of nitrogen fixed 50 mgm. With no nitrogen in the sand and only 12 mgm. in the seed, 70 mgm. were taken from the air. In working with water cultures, nitrates held down nitrogen fixation and nodule production. In the absence of nitrates the fixation varied from 17 to 83 mgm. per plant. He says that the nitrogen content of the soil influences the time at which the nodule empties itself. The presence of nitrogen in the soil brings this change at the time of seed formation, but its absence from the soil permits the process of nodule growth and nitrogen fixation to go on slowly during the life of the plant with an increase in it at the time of seed formation.

TABLE 1

Nitrogen fixed by peas grown in nutrient solutions containing nitrates
(Taken from Atwater)

	NUMBER OF EXPERIMENT	NITROGEN SUPPLIED			NITROGEN AT CLOSE			GAIN OR LOSS OF NITROGEN
		In seeds	In solution	Total	Vines, roots, etc.	Residual solution	Total	
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Group I. Small nitrogen ration	1	36.7	59.4	96.1	116.4	1.4	117.8	−21.7
	3	72.6	59.4	132.0	158.9	3.8	162.7	−30.7
	5	34.2	59.4	93.6	156.1	0.0	156.1	−62.5
	7	71.5	59.4	130.9	158.1	0.0	158.1	−27.2
	9	35.3	59.4	94.7	186.5	1.4	187.9	−93.2
	11	72.5	59.4	131.9	210.9	2.7	213.6	−81.7
Group II. Large nitrogen ration	2	34.4	136.9	171.3	178.9	2.0	180.9	−9.6
	4	75.2	136.9	212.1	200.6	12.8	213.4	−1.3
	6	34.8	136.9	171.7	149.6	1.2	150.8	−20.9
	8	70.3	136.9	207.2	197.5	12.7	210.2	−3.0
	10	34.6	136.9	171.5	277.8	35.7	313.5	−142.0
	12	68.8	136.9	205.7	260.2	45.7	305.9	−100.2

This work cited last, gives a different degree of effect for nitrates in a sand or open medium than it does for nitrates in water cultures. This fact may be of significance in explaining the injurious effects on nodule growth and nitrogen fixation which are attributed in many cases to the nitrates. The use of solutions may be inadequate for an experiment of this nature.

To test the effects of different forms of nitrogen on the nodule growth of legumes, Frank (14) used calcium nitrate, ammonium sulfate and urea. He analyzed seeds at the start and total plants at the close, and found that for the lupine the greatest growth and nitrogen increase in the plant, as well as the most profuse nodule production, took place in the absence of all nitrogenous compounds. The pea behaved similarly. He measured the nitrogen fixed in sand and soil by lupines, peas and red clover, obtaining the results given in table 2.

Frank's data show that the yellow lupine fixes less nitrogen on a humus soil than on a sand soil, or that soils with a higher nitrogen content fix less nitrogen with this legume. It may be possible that physical differences or other factors were responsible, for he gives no detailed description of relations other than nitrogen in the soil.

For the peas and clover the case is different. Both gave a decided increase in the soil rich in nitrogen. For the lupine he believes that its nitrogen-fixing power is less in a nitrogen-rich soil than in one very poor in this respect. Nevertheless, a soil already rich in nitrogen may be enriched in this element

TABLE 2

Nitrogen fixed in sand and soil by lupines, peas and red clover
(Taken from Frank)

KIND OF SOIL	NUMBER OF PLANTS	DRY WEIGHT HARVESTED	NITROGEN		INCREASE IN FOLD	NITROGEN IN SOIL		INFECTION
			In seed and inoculum	In harvest		Outset	Close	
Lupine								
		gm.	*gm.*	*gm.*		*per cent*	*per cent*	
Sand	5	14.76	0.035	0.3609	10.3	0.0096	0.0157	1 large nodule
Humus soil	4	23.32	0.0364	0.2816	7.7	0.1076	0.1208	1 to 7 nodules
Peas								
Humus soil	3	37.98	0.0282	0.7467	26.5	0.1076	0.1253	Of bean size
Red clover								
Sand		44.33	0.0457	0.7087	15.5	0.0073	0.0105	Rich in nodules
Sterilized sand not inoculated		7.18	0.0457	0.0687	1.5	0.0073	0.0079	Few nodules
Humus soil		222.02	0.0457	4.6406	105.5	0.1076	0.1184	Few nodules

by means of legumes. Peas and clover, he believes, reach their maximum fixing capacity only when using nitrogenous substances, especially nitrates, to supplement the bacteria on their roots, even though the direct opposite is true for the yellow lupine. The nitrogen enriching effect of legumes takes place not only in soils poor in nitrogen, but also in the better soils, rich in humus.

The above conclusion is quite the opposite to that of Maercker (31), who used the yellow lupine in sterilized and inoculated sand with varying amounts of potassium nitrate added. He found in this experiment that nitrates did not hinder or lessen the ability of the lupine as a nitrogen fixer. Nobbe and Hiltner (35) reported the diameter of *Robinia* nodules as 8 mm. in nitrogen-free soil, and 0.5 mm. in soil treated with nitrates.

Perhaps the most careful work in the early study of nitrogen fixation is that of Aeby (1) in his attempt to see if non-legumes would give nitrogen fixation. He used two soils, one a clay soil with 0.0783 per cent nitrogen and the other a "humus-rich" soil with 0.4050 per cent nitrogen. They were used in growing peas without nitrogen treatment, and with nitrogen added at four intervals to make a total of 2 gm. per pot of 4 kgm. of soil. Analyses were made for total nitrogen in all materials at the beginning and at the close. Any increase present at the close over that at the beginning represented fixation of nitrogen. This method of analysis gave a loss of nitrogen in fallow pots but a decided increase for those on which peas were grown. Peas growing in a rich soil fixed 1.976 gm. nitrogen per pot, while in the same soil treated with nitrogen, the fixation was but 1.621 gm. In the clay soil the corresponding figures are 2.759 gm. and 1.987 gm., respectively; a decrease of 0.355 and 0.772 gm. due to the nitrogen added. This indicates that in the soil rich, as well as one poor in nitrogen the addition of nitrogen depressed the fixation of atmospheric nitrogen. Furthermore, in the soil which was low in nitrogen the amount taken from the air by the peas was greater than in the rich soil, both when untreated and treated with nitrogen. Accordingly there was less fixation with increased amounts of nitrogen in the soils, which agrees with some of the preceding works cited. In the soil left fallow, he failed to recover as much nitrogen at the close as was present at the beginning. In the distribution of nitrogen in the roots and tops of plants, his results agree with those of Woods.

Nobbe and Hiltner (36) go farther in their statements than many others and conclude from a study of cross inoculation, that nodules have no influence on plant growth when plenty of soil nitrogen is available.

Salts containing nitrogen were used by Marchall (32) and found to inhibit nodule production in the following concentrations: alkaline nitrates 1 part in 10,000 and ammonium salts 1 part in 2000. By using soybeans and measuring the nitrogen increase in terms of the crop, when sterile or inoculated, or treated with nitrate nitrogen, Nobbe and Richter (38) found that with increased amounts of soluble nitrogen or humus substance added, the total nitrogen content of the crop decreased. They believed that small amounts of soluble nitrogen are beneficial to the young plants—at least until bacteroids are formed. According to them, inoculation was best in the absence of nitrates and decreased with the increase of the latter.

To judge the value of legumes as nitrogen fixers, Wohltmann and Bergene (51) used a variety of soils ranging in nitrogen from 0.046 per cent to 0.205 per cent and a peat soil with 1.650 per cent. These soils were treated with either ammonium nitrate or ammonium sulfate and planted to a number of legumes. They judged the amount of nitrogen taken from the air in terms of the number of nodules produced, and give the following results and conclusions in regard to the influence of the nitrogen in the soil on the amount of nitrogen fixed. On all soils to which ammonium nitrate was added the nodules failed to

develop and the plants grew well in the absence of them. Ammonium sulfate suppressed nodule production completely in nine cases and almost completely in two cases.

These men believe that legumes do not need the help of bacteria and atmospheric nitrogen when there is present in the soil an abundant supply of available nitrogen, and that they use soil nitrogen almost exclusively. According to these conditions, legumes used as green manure would not add nitrogen to that soil whose available supply of this essential element is high. They point out that it is the "available" nitrogen rather than the "total" nitrogen that has a detrimental influence on nitrogen fixation.

Similar results were obtained about 1904 by Nobbe and Richter (39) who undertook to determine the effect of soluble nitrogen in soil on the amount of nitrogen taken from the air by vetch. They measured fixation by difference between total nitrogen in plants that were inoculated and those that were not so treated. The increase in the nitrogen content caused by bacteria was

TABLE 3

Nitrogen in vetch, inoculated and uninoculated, treated with nitrate nitrogen
(From Nobbe and Richter)

	NO NITROGEN ADDED	500 MGM. NITROGEN ADDED	1000 MGM. NITROGEN ADDED
Inoculated (gm.)	1.533	1.887	2.295
Not inoculated (gm.)	0.095	0.389	0.618
Difference (gm.)	1.438	1.498	1.677
Increase (per cent of total)	93.8	79.38	73.07
Nitrogen ratio	1:16.1	1:4.8	1:3.7

considered as coming from the air. Increasing amounts of potassium nitrate were added to the soil and the entire plants analyzed. They found the results given in table 3.

According to their figures the effect of inoculation decreases with the increase in soluble soil nitrogen. Where no soluble nitrogen was added the bacteria increased the nitrogen in the plant sixteen-fold. As much as 93 per cent of the nitrogen in the inoculated legume came from the atmosphere. With the addition of 500 mgm. of soluble nitrogen the increase by bacteria was almost four-fold, or about 80 per cent of the nitrogen in the entire plant came from the air. When more nitrate was added, the depressive effect increased, though not proportionally. These amounts of soluble nitrogen, according to their data, decreased the action of the bacteria in supplying the plant with atmospheric nitrogen.

A large variety of nitrogenous compounds were tested by Flamand (12) for their influence on nodule production with peas, beans and vetch. He found that potassium nitrate prohibited nodule formation when used in amounts as low as 1 part in 10,000; sodium nitrate required but 1 part in

2000, or the equivalent of 1000 pounds per acre. Urea, oxamide, and potassium cyanide were very prejudicial to the production of nodules even when used in very dilute solution. Ammonium salts, either as nitrate or sulfate, prohibited nodules on vetch. Table 4 gives the proportions in which these compounds were fatal to nodule production.

This work was done in water cultures and as in previous works cited may have given more significant effects than would be true of soils.

The contention that assimilable nitrogen hinders nodule production is further supported by A. Hercke (20) who concludes that, "when the soil contains sufficient assimilable nitrogen, the presence of nodules on the roots has no influence on the nitrogen content of lupines. When soil is poor in nitrogen the presence of nodules increases the absolute as well as the percentage of nitrogen content of the plant." This is contradicted by S. Hercke (21) who found that nitrogen compounds as ammonium sulfate, potassium nitrate and asparagin favored the growth of nodule bacteria.

TABLE 4

Nitrogen concentrations fatal to nodule production
(From Flamand)

	Pisum sativum	*Vicia narbonensis*	*Faba equina*
Potassium nitrate	1:10,000	1:10,000	1:10,000
Sodium nitrate	1:10,000	1: 2,000	1: 2,000
Calcium nitrate	1: 2,000	1:10,000	1:20,000
Ammonium nitrate	1:10,000	1:20,000	1: 2,000
Ammonium sulfate	1:10,000	1:20,000	1:10,000

Fred and Graul (17) tested the effect of soluble nitrogenous compounds on nitrogen fixation in different kinds of soil and with different kinds of plants. In general, they found that increasing amounts of soluble nitrogen depressed nodule production, but for this effect a larger amount of soluble nitrogen was necessary than would probably ever occur under field conditions. They are supported in this by the field experiments of Ewart (11).

When it was found that bacteria are responsible for nitrogen fixation in conjunction with the plant, the question arose concerning their behavior in this respect independent of a host. Several researches have been carried out to see how nitrogen in various forms in the media influences the nitrogen-assimilating capacity of this organism when separated from the plant. In 1891 Beijerinck (4) found nitrogen fixation taking place by these bacteria (since named *Ps. radicicola*) independent of a plant, when the media contained ammonium, sodium, and potassium nitrates. Other early workers who reported fixation independent of the plant in the presence of nitrogenous compounds were Prazmowski (42), Berthelot (5), and Frank (14), but they said it was too small to be significant. Larger nitrogen fixation was reported

for the organism living independently by Maze (33) and by Lewis and Nicholson (29), the former finding as much as 23 mgm. of nitrogen fixed in 100 cc. of medium in 16 days.

The presence of nitrogen is considered injurious to the bacteria themselves according to Moore (34). He believes that, "the cultivation of bacteria upon media containing appreciable quantities of nitrogen for any length of time is sufficient to cause them to lose both the power of infection and that of fixing atmospheric nitrogen." This is strongly refuted, however, by Burrill and Hansen (7) who grew *Ps. radicicola* on nitrogen media for 30 months without a loss of its special adaptations or its capacity for producing nodules.

Very recently Fred (15) found that *Ps. radicicola* fixed more nitrogen when a trace of this element was present in the medium, but larger amounts of it retarded the process. He is supported by still more recent work of Hills (23) who found that fixation independent of the plant was increased by nitrate nitrogen. His results vary somewhat, and the fixation is large enough to be significant, which may not be wholly true of Fred's results.

This review of literature indicates that on the fixation of nitrogen by legumes, one may expect a significant influence from the nitrogen content of the soil. According to several researches soluble or assimilable nitrogen has a depressing influence, especially in water cultures and in soils when used in larger amounts. Nobbe and Hiltner (37) showed that the water-culture method itself was unfavorable for the development of nodules, and consequently nitrogen additions under such conditions may have been wrongly interpreted. The use of soil seems to be the best procedure and duplicates most nearly the field relations. Little has been done, however, in using carefully analyzed soil to measure the influence of soil nitrogen on the process of nitrogen fixation under those conditions generally prevailing in the soil. The opinions on this question are varied, though the more recent ones emphasize the assimilable or soluble nitrogen of the soil as being depressive to the efficiency of nitrogen-fixing bacteria. In most experiments cited the soluble nitrogen was applied as ammonia or nitrate salts and no account was taken of the nitrogen in organic matter. Such works fail to settle the significance of the organic matter in the soil concerning symbiotic nitrogen fixation.

III. EXPERIMENTAL

The following experimental work was undertaken in order to study further the relation of symbiotic nitrogen fixation to the nitrogen content of soils, especially the total nitrogen as obtained by usual soil analysis. In advising the use of legumes in a rotation cropping system, especially for the soils of the Corn Belt, which are moderately high in total nitrogen, the following questions often arise: Will the legumes fix any atmospheric nitrogen in a soil already containing large amounts of this element? If they do, how efficient will they be, and what significance does the nitrogen content of the soil have on the process? The following experimental work was undertaken with the hope of contributing a possible answer to these questions.

Plan of the experiment

In experimental work necessitating the measurement of nitrogen fixation, two general methods have been widely accepted. The first one consists in growing one set of legume plants without bacteria on the roots, and another set under like conditions but with the organism applied. The difference in the nitrogen content of the two sets of plants represents the increase due to bacteria. The second method consists of careful analyses of all materials to determine the total nitrogen present both at the outset and at the close of the experiment. Under carefully controlled conditions, with no nitrogen added, the increase of this element at the close over that at the beginning represents nitrogen drawn from the atmosphere.

Objections have been made to the first method on the ground that the soil or medium in which the plant grows is not taken into consideration. An increase in the nitrogen content of the plant because of the bacteria may mean that the plant was able to take more nitrogen from the soil and does not prove that all such increase in nitrogen came from the air.

The second method is more detailed and painstaking and gives difficulty because of lack of refined methods for determining total nitrogen. However, it measures the total nitrogen in all materials concerned at the beginning, and again at the close, so that the increase must come from some other source than the soil. In carefully controlled conditions this source must be the atmosphere. The latter method was used in this study.

The soil used for the pot cultures was a yellow silt loam from the unglaciated area of southern Illinois and contained 625 pounds of nitrogen per 2,000,000 pounds of surface soil. It was extremely low in organic matter, hence in such a poor physical condition that at first sight it would appear much like a clay or clay loam. It gave an acid reaction, and in order to neutralize this it was treated with calcium carbonate at the rate of 2 tons per acre. Plant nutrient elements other than nitrogen were added in soluble form during the time the plants were growing to assure good fertility, except for nitrogen which is the main deficiency in the soil (26). This soil was chosen because it was so low in nitrogen, and would offer a low nitrogen basis, which had become stable because of years of weathering, and which could be increased by several large increments without surpassing the nitrogen content of the more fertile soils.

Two kinds of legumes were grown, soybeans and cowpeas. The first series included soybeans but failed to do as well as expected in greenhouse work. They were replaced by cowpeas for the later series which were grown out-of-doors during most of the time. Insects molested the plants in all series and were combatted with chemicals containing no nitrogen. Red spiders were especially prevalent and were sprayed with cold water or potassium sulfide solution. During the first series a fungus gnat (*Sciaria mycetophilidae*) (18) infested the soils which were high in organic matter. Later troubles from this insect were not experienced. Insect infestations were prevalent on all plants,

so that even though they may have hindered growth to some extent, their disturbances would not prevent comparable results.

In the first series which contained soybeans, four duplicate series of 1-gallon pots were used with the soil treatments given in table 5.

TABLE 5

Soil treatments in crop series 1
(Soybeans)

POT	NITROGEN ADDED TO SOIL (POUNDS PER 2,000,000 POUNDS OF SOIL)
	Pot series 1
1–2	None (not inoculated)
3–4	None
5–6	10 pounds as nitrate
7–8	50 pounds as nitrate
9–10	150 pounds as nitrate
	Pot series 2
11–12	1000 pounds as clover tops
13–14	2000 pounds as clover tops
15–16	3000 pounds as clover tops
17–18	4000 pounds as clover tops
19–20	5000 pounds as clover tops
	Pot series 3
21–22	1000 pounds as clover tops and 10 pounds as nitrate
23–24	2000 pounds as clover tops and 10 pounds as nitrate
25–26	3000 pounds as clover tops and 10 pounds as nitrate
27–28	4000 pounds as clover tops and 10 pounds as nitrate
29–30	5000 pounds as clover tops and 10 pounds as nitrate
	Pot series 4
31–32	1000 pounds as clover tops and 50 pounds as nitrate
33–34	2000 pounds as clover tops and 50 pounds as nitrate
35–36	3000 pounds as clover tops and 50 pounds as nitrate
37–38	4000 pounds as clover tops and 50 pounds as nitrate
39–40	5000 pounds as clover tops and 50 pounds as nitrate

The addition of nitrate nitrogen and clover tops was made on the basis of one acre, or 2,000,000 pounds of soil. In all cases a constant amount of soil was used, and the increasing amounts of clover tops added gave increasing amounts of substrate for the plants in the series. This treatment also brought about decided changes in the soil's physical condition. Four pots were used as checks. All pots in the series were inoculated except two of the check pots, no. 3 and 4, which remained uncontaminated and showed no nodules at the close of the experiment.

TABLE 6

Soil treatments in crop series 2

(Cowpeas)

POT	NITROGEN ADDED TO SOIL (POUNDS PER 2,000,000 POUNDS OF SOIL)
	Pot series 1
1a–2a	None
3a–4a	10 pounds as nitrate
5a–6a	25 pounds as nitrate
7a–8a	50 pounds as nitrate
9a–10a	100 pounds as nitrate
	Pot series 2
1–2	None (not inoculated)
3–4	None
11–12	1000 pounds as clover tops
13–14	2000 pounds as clover tops
15–16	3000 pounds as clover tops
17–18	4000 pounds as clover tops
19–20	5000 pounds as clover tops

TABLE 7

Soil treatments in crop series 3

(Cowpeas)

POT	NITROGEN ADDED TO SOIL (POUNDS PER 2,000,000 POUNDS OF SOIL)
	Pot series 1
1b–2b	None
3b–4b	50 pounds as nitrates
5b–6b	100 pounds as nitrates
7b–8b	150 pounds as nitrates
9b–10b	200 pounds as nitrates
11b–12b	250 pounds as nitrates
13b–14b	50 pounds as nitrates (added at intervals)
	Pot series 2
1–2	None
3–4	None
11–12	1000 pounds as clover tops
13–14	2000 pounds as clover tops
15–16	3000 pounds as clover tops
17–18	4000 pounds as clover tops
19–20	5000 pounds as clover tops

The second series was seeded with cowpeas, and contained two pot series, one treated with nitrates, and one with organic matter. Original soil, the same as used previously, was treated with nitrate nitrogen, while the series treated with organic matter consisted of a part of those soils used in the first crop series The four check pots and the ten pots that had been treated with nitrogen as clover tops only, were taken to complete this series. These soils were in better physical condition and the stage of most rapid decay seemed to have been passed. Constant amounts of soil (3300 gm. water-free soil) were weighed into each pot. All pots were inoculated except the two check pots, but evidently these were already inoculated, or became contaminated, for nodules were present at the close of the series. This crop series with its treatment is summarized in table 6.

The third series was similar in soil treatment to that of the second. Some of the original soil was treated with nitrates for one pot series, while those soils which had been used in both previous series were used for the other pot series. Cowpeas were grown as the crop. The treatments are given in table 7.

The last two series in which cowpeas were grown were kept out-of-doors near the greenhouse for much of the time. This seemed to favor better growth, and lessened the insect troubles. Care was taken to move the plants inside before rain and only once did rain fall on each series. It was only a slight shower and the effects in adding nitrogen were negligible.

Analytical methods

The usual analytical methods were employed. To measure the total nitrogen in organic matter such as seeds and plants, the materials were digested with mercury and sulfuric acid or with acid and sodium sulfate, neutralized with sodium hydroxide and potassium sulfide, distilled into standard acid and titrated with standard alkali. For the total nitrogen determination on soils, 10-gm. samples were first dried for 8 hours at a temperature of 107°–108°C., the loss in moisture determined, and then transferred for digestion. Sulfuric acid containing salicylic acid was added and allowed to stand for some time, usually over night. Sodium thiosulfate was introduced and heated slowly until frothing ceased. Mercury was then added and digested to clearness, potassium permanganate being used to assure complete oxidation. This was then neutralized with alkali and distilled into standard acid, according to the usual procedure.

For the determination of nitrate nitrogen, samples of soil were dried at 107°–108°C. for 8 hours and then extracted with 0.0625 *N* hydrochloric acid. After being made alkaline, the ammonia was boiled off, and the original volume replaced by nitrogen-free water. Devarda's metal was added, the reduced nitrogen distilled into 0.0357 *N* standard sulfuric acid and titrated.

In all the determinations the greatest care was exercised. The organic matter was dried, weighed, thoroughly ground, and kept in air-tight containers to prevent decided variations in moisture content before an entire series could be analyzed. Purest chemicals were used and all reagents were made up in large amounts to insure greater uniformity in results. Standard acid for nitrogen in soil was of 1/14 normality and with sodium alizarin sulfonate as an indicator, titrations checked within 0.2 cc., a variation equivalent to 0.2 mgm. nitrogen in 10-gm. samples, or 40 pounds of nitrogen in 2,000,000 pounds of soil. For nitrate determinations, a sulfuric acid of 1/28 normality was used and 0.2 cc. allowed as variation in duplicates. Determinations in moisture loss for 10-gm. samples were usually less than 6 mgm. All calculations and determinations for soil were on the water-free basis to insure uniformity. Samples were run in triplicate and quadruplicate to offset errors. Only doubly distilled nitrogen-free water was used, both in analytical work and in the pot cultures.

Discussion of crop series

Series 1 (Soybeans)

The soil for this series was thoroughly mixed in a quantity large enough for all the pots. To determine the nitrogen in it, a large sample was taken and ground to pass a 100-mesh sieve and then analyzed for total nitrogen. According to the analysis, 30-gm. quantities of air-dry soil, which were equivalent to 29.1970 gm. water-free soil, contained 9.1568 mgm., or 0.03136 per cent nitrogen on the latter basis, as an average of five determinations varying less than 0.2 mgm. Each pot received 3500 gm. of soil with a moisture content of 14.01 per cent, or the equivalent of 3421 gm. of water-free soil, and a nit ogen content according to the above analysis of 1072 mgm.

For one pot series the soil was treated with nitrate nitrogen only, at the rate of 17.2, 86.0 and 258 mgm. of nitrogen per pot, or the equivalent of 10, 50 and 150 pounds per 2,000,000 pounds of soil. For three additional pot series the nitrogen in the soil was increased at increments equivalent to 1,000 pounds of nitrogen per 2,000,000 pounds of soil by means of finely ground clover tops. The clover tops contained 2.709 per cent of nitrogen, as an average of 12 analyses. With 3500 gm. of soil per pot an increment of 1000 pounds per 2,000,000 —one part in 2000—required 1.75 gm. of nitrogen, or 64.6 gm. of clover tops. Accordingly, for the second pot series enough clover tops were added to give a series of the original soil, whose nitrogen was increased at the rate of 1000, 2000, 3000, 4000 and 5000 pounds per acre. The third series was similar to the second except that in addition to the organic matter sodium nitrate also was added to all the pots at the rate of 10 pounds of nitrogen per 2,000,000 pounds of soil, while the fourth series differed from the third only by the addition of sodium nitrate at the rate of 50 pounds of nitrogen instead of 10. This gave one pot series whose nitrogen was increased as nitrate, one with increas-

ing nitrogen in organic matter as clover tops, another increasing with clover tops and 10 pounds of nitrate nitrogen and the fourth whose nitrogen increased as clover tops and 50 pounds of nitrate nitrogen. The addition of varying amounts of clover tops to 3500 gm. of soil increased the amount of soil in the pots, so that the weight of soil in the different pots was not a constant figure.

According to Hilgard (22) and others, the best moisture content of soils for proper plant development is one-half of the moisture-holding capacity. On this basis, determinations of moisture-holding capacity were made on these soil series as modified by additions of clover tops, and the water applied to the pots in quantities to give the equivalent of 50 per cent of the moisture-holding capacity. An attempt was made to maintain such conditions throughout the experiment by weighing the pots at intervals, but the labor involved was large and the growth of the crops prohibited absolute accuracy. It was therefore necessary to apply the water according to one's best judgment. The presence of large amounts of organic matter caused trouble in keeping the moisture at optimum amounts.

Soybean seeds were selected so that each seed weighed 140 mgm. Six of these were planted in each pot, in order that five vigorous plants might be assured in spite of low vitality and poor germination. The sixth plant or seed was removed after sufficient time had elapsed for the plants to get well started. According to the analyses of the beans, each seed contained an average of 9 mgm. of nitrogen.

The growth of this series was fair. Numerous replantings were necessary because the large amounts of fresh organic matter interfered with proper germination of the seeds, a difficulty experienced also by Fred (16) at Wisconsin and by others at Illinois with oats, cowpeas and clover after turning under alfalfa. The injurious effects were more marked with the increase in organic matter so that on the pots receiving clover tops at the rate of 5000 pounds of nitrogen per 2,000,000 pounds of soil, only small plants were produced. These effects were not offset by the nitrate nitrogen added at the rate of 10 pounds and 50 pounds per 2,000,000 along with the organic matter. Some plants "damped-off" at the age of four weeks, while others died later. The growth was much influenced by the organic matter in the soil, being the best of the entire series where 1000 and 2000 pounds of nitrogen had been added as clover tops, but decidedly poorer in soil with larger amounts of this material added. The plants showed a less deep color, smaller leaves, less branching and more translocation, with the increase in organic matter above the equivalent of 2000 pounds of nitrogen per acre.

The effect of the nitrates was marked in the soil when these were added alone, but gave no appreciable effect when coupled with the organic matter. In the soil alone, the increasing amounts of nitrates gave improved plant growth for the early part of the plant season, but these differences were obliterated when the crop matured, as was shown by the weights and nitrogen content of the crop. The differences in the four pot series just before their close are shown in plate 1.

Nitrates in soil growing soybeans. Before removing the plants at harvest, samples of soil were taken and nitrates determined by extraction and reduction with Devarda's alloy. Forty-gram samples of water-free soil were used. Determinations on those pots with organic matter added were somewhat erratic and failed to check well in duplicate. Since large amounts of organic matter are known to interfere with reduction methods (9, 48) and give high results, full credence cannot be placed on the results from these soils with organic matter. Table 8 gives the results of the determinations of nitrates.

TABLE 8

Nitrate nitrogen in soil growing soybeans

POT	NITROGEN ADDED (POUNDS PER 2,000,000 POUNDS OF SOIL)	MILLIGRAMS IN 40 GRAM SOIL	MILLIGRAMS PER POT	POUNDS PER 2,000,000 POUNDS OF SOIL
1	None	0.337	28	16.8
2	None	0.366	31	18.3
3	None	0.378	32	18.9
4	None	0.337	28	16.8
5	10 pounds as nitrate	0.397	33	19.8
6	10 pounds as nitrate	0.378	32	18.9
7	50 pounds as nitrate	0.337	28	16.8
8	50 pounds as nitrate	0.397	33	19.8
9	150 pounds as nitrate	1.353	115	67.6
10	150 pounds as nitrate	1.533	131	76.6
11	1000 pounds as clover tops	2.031	176	100.0
12	1000 pounds as clover tops	0.816	71	40.0
13	2000 pounds as clover tops	2.442	216	122.0
14	2000 pounds as clover tops	5.835	516	291.0
15	3000 pounds as clover tops	15.852	1425	792.0
16	3000 pounds as clover tops	12.585	1132	629.0
17	4000 pounds as clover tops	9.459	864	472.0
18	4000 pounds as clover tops	9.757	892	487.0
19	5000 pounds as clover tops	8.403	780	420.0
20	5000 pounds as clover tops	8.722	810	436.0

From the above table it is evident that the pots receiving the equivalent of 10 and 50 pounds of nitrate nitrogen per acre contained no more nitrogen in this form at the close of the series than the pots left untreated. Evidently the plants consumed all that was applied, since soil conditions were unfavorable to denitrification, the other possible chance for nitrate removal. With the pots receiving the equivalent of 150 pounds per acre the conditions were different and the nitrate content was much higher. The application of such a large amount, far above that needed for the plant growth, left some in the soil, since no leaching took place and the open soil prohibited denitrification. This would suggest that such a large application supplies more nitrogen than a single crop of soybeans can remove.

In the soils with organic matter the figures indicate excessive amounts of nitrates, reaching the maximum in the pots receiving 3000 pounds of nitrogen as clover tops per 2,000,000 pounds of soil, and decreasing somewhat with the heavier applications. The high results may have been caused partly by the interference of the organic matter.

Nodule production by soybeans. When the plants were harvested, the roots were removed from the soil as completely as possible and studied for nodule production. Pots 1 and 2, untreated, which had not been inoculated at the outset were still sterile in respect to the soybean bacteria, containing no nodules and showing no contamination from the inoculated pots all about them. This indicates that there is no great danger of inoculation by contamination in an experiment of short duration of this kind with soybeans when no special precautions are taken. In the pots in this series, treated with sodium nitrate, many good-sized nodules were present giving no visible effect from the nitrate treatment either on the size or on the number of the nodules, except perhaps in the two pots receiving the equivalent of 150 pounds of nitrogen per acre. In these the nodules were smaller, but this difference was offset by increased numbers.

In the pot series treated with clover tops, nodule production decreased with added amounts of organic matter. However, in the soils with the highest application there was such a poor development of the plants that very few nodules could be expected. The low vitality and poor plant growth rather than any disturbing factors in the soil may have been the cause of insignificant nodule formation. There was no evidence to indicate that with healthy plants nodule production would have been prohibited by these large amounts of organic matter.

In the soil treated with such large amounts of clover tops as would give 5000 pounds of nitrogen in 2,000,000 pounds of soil, the original composition and physical make-up were radically altered. Such modifications of soil condigions invited the infestation by a winged fungus gnat (*Sciara mycetophilidae*) which may have consumed and removed much of the nitrogen. The open soil structure and a plentiful food supply in the form of decaying organic matter furnished an excellent habitat for these small insects. The infestation originated in the pots with the heaviest applications of organic matter, but spread to all pots so treated. Its duration was limited to a certain stage or period in the decomposition of the organic matter, and the insects were present only long enough to develop the larvae and pupae of one generation. They may have been a disturbing factor of which no account could be taken, and may have been partly responsible for the losses of nitrogen in these series.

In tables 9, 10, 11 and 12 is given the nitrogen balance for the four pot series with soybeans. Many of the analytical data are omitted and only the summation figures are given to show the balance between the total nitrogen in the soil and seed at the beginning, and that of the soil and crop at the close. Figures given are the results of quadruplicate determinations in the soil, while in

the crop the total material was analyzed. Four pots given in the first series are used as the checks for the remaining pot series.

Following the tables is a graphical representation of the increases and losses in total nitrogen shown in the tables. The ordinates represent milligrams of nitrogen gained or lost while the abscissas represent nitrate nitrogen added to the soil in case of one curve, and the total nitrogen present in the soil at the outset for the remaining curves.

Instead of showing an increase in nitrogen, most pots show a decrease. Increases in nitrogen, when they occur, are insignificant as compared with the

TABLE 9

Nitrogen balance—Soybeans

Pot series 1, soil treated with nitrates

POT	NITRATE NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	CROP WEIGHT	NITROGEN IN CROP	NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE OR FIXATION	AVERAGE
	mgm.	*gm.*	*mgm.*	*mgm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None (uninoculated)	9.8394[a]	2.8713[a]	998[b]	2.9	36[c]	1034	1117[d]	−83	−36
2	None (uninoculated)	9.8215	2.9714	1032	4.0	58	1090	1117	−55	
3	None	9.8260	2.9848	1040	3.5	98	1138	1117	21	
4	None	9.8288	2.8864	1003	3.85	89	1092	1117	−25	
5	17.2	9.7864	2.8783	1005	3.85	95	1100	1134	−34	−16
6	17.2	9.8025	2.9714	1037	4.75	98	1135	1134	− 1	
7	86	9.8029	2.9140	1016	5.12	113	1129	1203	−74	−93
8	86	9.8183	2.8498	992	4.25	98	1090	1203	−113	
9	258	9.8087	3.2243	1124	4.25	124	1248	1375	−127	−122
10	258	9.8190	3.1790	1106	3.70	151	1257	1375	−118	

[a] Average of four determinations, or duplicates made at two different times.

[b] Based on 3421 gm. water-free soil in each pot.

[c] Found by analyzing entire crop, roots and tops.

[d] Based on the analyses of the soil at the outset giving 0.03136 per cent of nitrogen, or 1072 mgm. of nitrogen in each pot with 3421 gm. of soil, and 5 seeds containing 45 mgm. of nitrogen. Additions of nitrate were equivalent to 17.2, 86 and 258 mgm. of nitrogen.

losses. When the soil was treated with nitrates only, one of the duplicate pots showed an increase in nitrogen at the close over that present at the outset, but this increase was within the limit of difference between duplicate determinations which, when calculated per pot, was 60 mgm. of nitrogen. The limits of error in nitrogen per pot were less, however, than this figure, as a result of quadruplicate analysis. The largest loss of nitrogen was 127 mgm. As more nitrate nitrogen was added at the beginning, the loss at the close increased, corresponding closely to the amount added. This indicates that perhaps the

analytical procedure failed to detect the nitrate nitrogen added. Had the nitrate nitrogen been incorporated into the plant tissue as protein, it would certainly have been found; but it might be possible, in spite of all care in this respect, that such small amounts of nitrate were not detected by the analysis for total nitrogen at the close. Later crop series receiving far larger amounts of nitrogen as nitrate, failed to show any such discrepancies.

TABLE 10

Nitrogen balance—Soybeans

Pot series 2, soil treated with clover tops

POT	NITROGEN ADDED AS CLOVER TOPS	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	SOIL IN POT	NITROGEN IN POT AT CLOSE	CROP WEIGHT	NITROGEN IN CROP	NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
	mgm.	*gm.*	*mgm.*	*gm.*	*mgm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
11	1750[a]	9.8361[b]	7.7461[b]	3480	2740[c]	2.85	122	2862	2867	−5	79
12	1750	9.8385	8.0541	3480	2848	4.70	182	3030	2867	163	
13	3500	9.8279	12.3984	3539	4464	4.15	189	4653	4617	36	90
14	3500	9.8216	12.7212	3539	4583	4.05	179	4762	4617	145	
15	5250	9.7951	15.4721	3598	5683	3.55	199	5882	6367	−485	−351
16	5250	9.7905	16.3141	3598	5995	2.70	154	6149	6367	−218	
17	7000	9.7815	19.9884	3657	7473	1.85	131	7604	8117	−513	−673
18	7000	9.8248[f]	19.3026	3657	7184	1.70	99	7283	8117	−834	
19	8750	9.7879	23.2323	3716	8820	1.25	91	8911	9867	−956	−951
20	8750	9.8146	23.3436	3716	8838	1.20	82	8920	9867	−947	

[a] Clover tops added were figured on the basis of 3500 gm. of soil per pot instead of 3421, hence are not truly equivalent to the number of pounds per 2,000,000 pounds of soil as the increments were intended.

[b] Average of four determinations, or duplicates made at two different times.

[c] Based on water-free soil in pot as given in fifth column.

[d] Found by analyzing entire crop, roots and tops.

[e] Based on the analyses of the soil at the outset giving 0.03136 per cent of nitrogen, or 1072 mgm. of nitrogen in each pot with 3421 gm. of soil, and 5 seeds containing 45 mgm. of nitrogen.

[f] Two determinations only.

In the three series receiving organic matter alone and organic matter in conjunction with 10 and 50 pounds of nitrogen as nitrate, there was an increase in nitrogen for all pots receiving clover tops equal to 1000 pounds of nitrogen per acre, and for two pots treated with organic matter equivalent to 2000 pounds of nitrogen. All other pots in these three series showed decided losses. In the pots with the equivalent of 5000 pounds of nitrogen added as clover tops this loss ran as high as 1150 mgm. of nitrogen in some few cases.

These large losses must have resulted from the very rapid decomposition of the fresh organic matter and doubtless the nitrogen of the clover tops escaped as gaseous ammonia. The excessive application encouraged rapid decomposition, and with 320 gm. of clover tops incorporated in 3421 gm. of soil, the proportion of soil may have been too small to absorb all the ammonia. The losses increased fairly regularly with larger applications of clover tops, though in no definite mathematical relation.

TABLE 11

Nitrogen balance—Soybeans

Pot series 3, soil treated with clover tops and 10 pounds of nitrate nitrogen

POT	NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	SOIL IN POT	NITROGEN IN POT AT CLOSE	CROP WEIGHT	NITROGEN IN CROP	NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
	mgm.	*gm.*	*mgm.*	*gm.*	*mgm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
21	1750[a] as clover tops and	9.7732[b]	7.9007[b]	3480	2813[c]	3.85	144[d]	2957	2884[e]	73	125
22	17.2 as nitrate	9.7804	8.1348	3480	2894	4.1	167	3061	2884	177	
23	3500 as clover tops and	9.7684	11.8428	3539	4290	4.2	167	4457	4634	−177	−116
24	17.2 as nitrate	9.7537	12.1518	3539	4409	3.9	169	4578	4634	−56	
25	5250 as clover tops and	9.7672	14.8770	3598	5480	2.2	123	5603	6384	−781	−616
26	17.2 as nitrate	9.7629	15.7567	3598	5806	2.0	126	5932	6384	−452	
27	7000 as clover tops and	9.7648	18.6387	3657	6980	1.9	105	7085	8134	−949	−876
28	17.2 as nitrate	9.7642	19.2363	3657	7204	1.85	126	7330	8134	−804	
29	8750 as clover tops and	9.7626	22.6532	3716	8628	0.05	73	8701	9884	−1183	−1152
30	17.2 as nitrate	9.7556	22.4843	3716	8653	1.80	110	8763	9884	−1121	

[a] Clover tops and nitrate added were figured on the basis of 3500 gm. of soil per pot instead of 3421, hence are not truly equivalent to the number of pounds per 2,000,000 pounds of soil as the increments were intended.

[b] Average of four determinations, or duplicates made at two different times.

[c] Based on weights of soil given in fifth column.

[d] Found by analyzing entire crop, roots and tops.

[e] Nitrogen in soil 1072 mgm., nitrogen in seed 45 mgm., nitrogen in 64.59 gm. clover tops equivalent to 1000 pounds per acre 1750 mgm., and nitrogen as nitrate 17.2 mgm.

From the data of this series, one cannot say with any great certainty that the soybeans so grown used atmospheric nitrogen. The treatment of nitrate apparently prevented an increase in nitrogen while the treatment of clover tops equal to 1000 pounds of nitrogen gave an increase of nitrogen. This must

have come from the air in the form of nitrogen fixation. The fact that there is a loss of total nitrogen in the system does not deny the possibility of the plants fixing nitrogen, for the legume may have used atmospheric nitrogen at the same time this escape from the soil was taking place.

The crop growth on all these series was small, and the total nitrogen in the crop of any pot never exceeded 200 mgm. With such small plant growth no

TABLE 12

Nitrogen balance—Soybeans

Pot series 4, soil treated with clover tops and 50 pounds of nitrate nitrogen

POT	NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	SOIL IN POT	NITROGEN IN POT AT CLOSE	CROP WEIGHT	NITROGEN IN CROP	NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
	mgm.	*gm.*	*mgm.*	*gm.*	*mgm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
31	1750[a] as clover tops and	9.7603[b]	8.4575[b]	3480	3015[c]	3.6	154[d]	3169	2953[e]	216	114
32	86 as nitrate	9.7656	7.9314	3480	2826	3.3	140	2966	2953	13	
33	3500 as clover tops and	9.7694	12.0295	3539	4357	3.25	144	4501	4703	−202	−187
34	86 as nitrate	9.7561	12.0698	3539	4378	3.05	152	4530	4703	−173	
35	5250 as clover tops and	9.7481	15.0377	3598	5550	1.5	93	5643	6463	−820	−490
36	86 as nitrate	9.7474	16.7461	3598	6181	1.9	122	6303	6463	−160	
37	7000 as clover tops and	9.7386	19.6224	3657	7368	1.5	101	7469	8203	−734	−950
38	86 as nitrate	9.7546	18.4956	3657	6934	1.65	103	7037	8203	−1166	
39	8750 as clover tops and	9.7601	23.3127	3716	8875	0.9	47	8922	9953	−1031	−1148
40	86 as nitrate	9.7570	22.6049	3716	8609	1.05	79	8688	9953	−1265	

[a] Clover tops and nitrate added were figured on the basis of 3500 gm. of soil per pot instead of 3421, hence are not truly equivalent to the number of pounds per 2,000,000 pounds of soil as the increments were intended.

[b] Average of four determinations or duplicates made at two different times.

[c] Based on weights of soil given in fifth column.

[d] Found by analyzing entire crop, roots and tops.

[e] Nitrogen in soil 1072 mgm., nitrogen in seed 45 mgm., nitrogen in 64.59 gm. clover tops equivalent to 1000 pounds per acre 1750 mgm., and nitrogen as nitrate 86 mgm.

great fixation was possible for the fixation of atmospheric nitrogen consists in the utilization of this form of nitrogen by the plant for tissue building, and unless plant growth is significant, no marked use of atmospheric nitrogen can be expected. The conditions of this part of the experiment gave too narrow a margin between the possible nitrogen fixed and the limits of variation, and

even though increases were shown in some cases, they failed to give assurance of any significant fixation. Unless a reasonable amount of fixation was taking place, the influence of the amount of nitrogen in the soil upon it could not be measured.

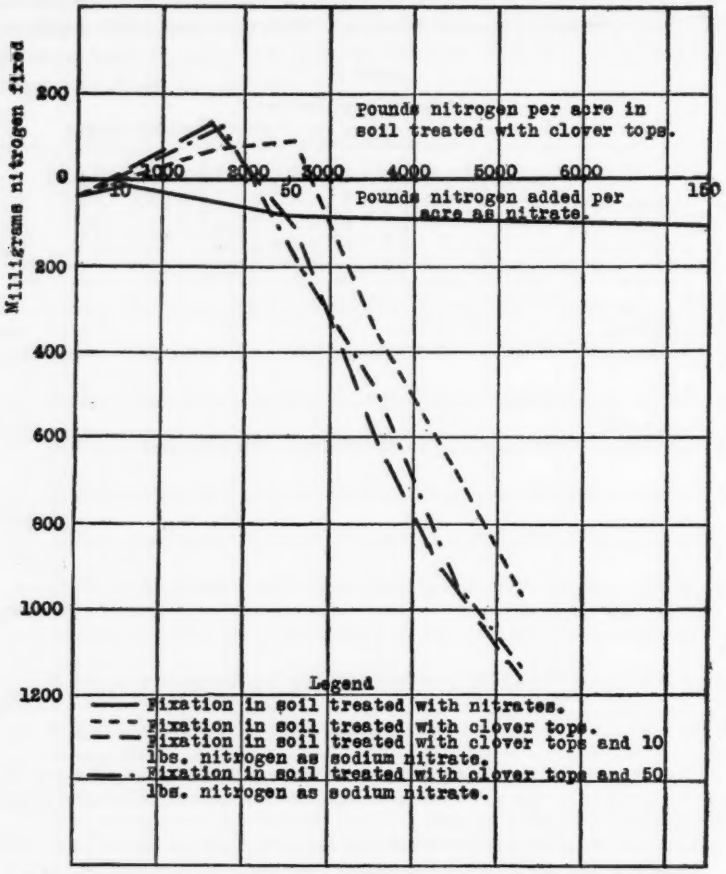

FIG. 1. GRAPHS SHOWING NITROGEN FIXATION BY SOYBEANS ON SOILS TREATED WITH NITRATES, CLOVER TOPS, AND WITH NITRATES AND CLOVER TOPS

Series 2 (Cowpeas)

For the second crop series cowpeas were grown in the hope that they would be less subject to the infestation by insects, more apt to do well under greenhouse conditions and more able as nitrogen fixers. Only two soil treatments were employed, one in which increasing amounts of nitrate nitrogen were

added and the other one in which the nitrogen had been increased by organic matter.

Some of the original soil left over when the previous crop series was made up and stored in the dry condition was used for the treatment with nitrate nitrogen. The equivalent of 3300 gm. of water-free soil was weighed into each pot and enough sodium nitrate added in a solution to give increases in nitrogen equivalent to 10, 25, 50, and 100 pounds of nitrogen in 2,000,000 pounds of soil.

For the series whose nitrogen increase was in the form of organic matter, the soils used in the previous series in the pots 1 to 4 and 11 to 20, inclusive, were used again. Pots 1 to 4 had received no treatment with nitrogen and again served as checks. Pots 11 to 20 were also used without modification, save that the amount of soil in each pot was limited to 3300 gm. on the water-free basis. The nitrogen content in these soils no longer showed increments of 1000 pounds per 2,000,000 pounds of soil as a result of losses while growing soybeans, but were used because their differences in nitrogen content were very marked and they were similar in all respects except this one.

Plant-foods other than nitrogen were supplied in a soluble form. Calcium carbonate was added at the rate of 2 tons per 2,000,000 pounds.

Six cowpeas of uniform weight and known nitrogen content were planted in each pot and later when the plants were well started were reduced to five. The nitrogen added by the five cowpea seeds was equivalent to 43 mgm. During the early spring the pots were kept in the greenhouse but later were kept outdoors in a screened area. No difficulty in germination was experienced, and the growth was decidedly better than that of the soybeans.

Differences in these two series were soon noticeable. For the series receiving nitrate the plants were taller, the leaves larger and the color deeper where greater amounts of nitrate were added. These differences later disappeared so that by the time of harvest, there were no significant variations within the entire series, either in crop weight or total nitrogen.

The soil series treated with nothing but organic matter and which had been previously used for soybeans, gave a decidedly better growth of cowpeas than the series treated with nitrates. The differences are shown in plates 2 and 3. In this series the early growth was best in the pot receiving 1000 pounds of nitrogen per acre as clover tops. There was a decrease in growth with the increase of added organic matter, but even all these were equal to the check, receiving no organic matter. In the latter part of the growing season these differences were reversed, and the largest crop yields and the largest total amounts of nitrogen in the crop were produced on the pots receiving most organic matter. The crops were grown for about 135 days and then harvested. Some plants had produced blossoms and a few had set pods. The fact that the pots were much shaded by some large trees lengthened the vegetative period and delayed seed development.

When the crops were harvested the roots were removed as completely as possible and carefully examined for nodules. It is important to note that the nitrate series produced many nodules, even with 100 pounds of nitrogen in this form. Where organic matter was added, the nodules were most numerous in the lesser applications but were larger in size as the applications increased. There were no indications in any pots that insufficient nodules were present for nitrogen fixation. If the presence of the nodule is certain evidence that atmospheric nitrogen is used by the plant, then both these soil series permitted fixation to take place in all pots regardless of treatments.

Determinations of nitrate nitrogen in the soils in all the pots were made just before the plants were harvested. In the soil treated with sodium nitrate, no nitrogen in this form was found. The determinations of nitrates gave re-

TABLE 13

Nitrogen balance—Cowpeas I

Pot series 1, soil treated with nitrates

POT	NITRATE NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	WEIGHT OF CROP		NITROGEN IN THE CROP		NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
					Tops	Roots	Tops	Roots				
	mgm.	*gm.*	*mgm.*	*mgm.*	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1a	None	9.7673[a]	3.2755[a]	1106[b]	15.75	8.855	415	152	1673	1077[c]	596	688
2a	None	9.6763	3.7410	1275	14.75	9.435	418	164	1857	1077	780	
3a	16.5	9.7201	3.3414	1134	14.80	7.820	306	139	1579	1093	486	533
4a	16.5	9.7034	3.1594	1074	16.40	7.800	440	159	1673	1093	580	
5a	41.0	9.6933	3.1163	1061	14.45	7.145	292	147	1500	1118	382	512
6a	41.0	9.7568	3.3418	1130	17.65	7.781	445	185	1760	1118	642	
7a	82.0	9.7691	2.9373	992	16.15	8.408	404	162	1558	1159	399	453
8a	82.0	9.7215	3.1031	1053	16.65	7.675	445	168	1666	1159	507	
9a	157.0	9.7441	3.0102	1019	15.45	6.798	347	124	1490	1234	256	264
10a	157.0	9.7641	2.9108	983	14.80	5.610	385	138	1506	1234	272	

[a] Average of three determinations.

[b] Calculated on the basis of 3300 gm. of water-free soil.

[c] Based on 3300 gm. of water-free soil with 0.03136 per cent of nitrogen, or 1077 mgm. of nitrogen, and five cowpea seeds with 43 mgm. of nitrogen.

sults no larger than those of the blank determinations. For the series with the organic matter, this treatment interfered with the analytical procedure for nitrates so that no definite statement of the amounts of nitrate present can be made. Results were erratic, but indications pointed to the presence of comparatively large amounts of nitrate nitrogen. Evidently nitrification was going on and nitrates were present in the soil but this had not prohibited the production of many large-sized nodules on the roots of the cowpeas.

The harvested crops were thoroughly dried, weighed and later ground to permit uniform sampling. The crop growth was too large to be analyzed in total and only triplicate 2-gm. samples were used. The soil was air-dried,

ground and sampled. Analyses were made by the same methods used in the previous series, but instead of using four samples, only triplicate determinations were made. On the basis of these analyses, the total nitrogen in the soil and the crop at the close of the series was calculated.

Tables 13 and 14 give the nitrogen balance for the second crop series with cowpeas. Only the summations of analytical data are given. Since the soils in pots 1 to 4 and 11 to 20 were analyzed at the close of the soybean series no analyses were made on these soils at the beginning of this series, but the amount

TABLE 14

Nitrogen balance—Cowpeas I

Pot series 2, soil treated with clover tops

POT	NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	WEIGHT OF CROP		NITROGEN IN THE CROP		NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
					Tops	Roots	Tops	Roots				
	lbs.	*gm.*	*mgm.*	*mgm.*	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	9.7140[a]	3.5308[a]	1199[b]	21.70	6.976	604[c]	135[d]	1938	1006[e]	932	877
2	None	9.7324	3.4744	1178	19.65	6.040	559	126	1863	1041	822	
3	None	9.7423	3.3086	1120	18.75	7.170	504	157	1779	1045	734	749
4	None	9.7026	3.2257	1097	21.90	10.182	502	178	1777	1012	765	
11	995[f]	9.7421	6.8328	2314	34.75	11.705	1066	201	3581	2641	941	913
12	995	9.7419	7.0649	2393	35.30	9.525	1032	205	3630	2744	886	
13	1960	9.7324	9.5746	3246	33.00	10.550	977	192	4415	4206	209	234
14	1960	9.7412	9.5912	3249	39.75	12.711	1125	209	4576	4317	259	
15	2890	9.7462	11.5307	3904	39.20	11.959	1217	204	5325	5255	70	−81
16	2890	9.7514	11.6965	3958	36.45	12.270	1124	226	5308	5541	−223	
17	3790	9.7549	13.6027	4601	43.65	9.975	1256	153	6010	6786	−776	−632
18	3790	9.7468	13.4631	4558	37.25	8.347	1290	189	6037	6526	−489	
19	4660	9.7461	15.6151	5287	45.70	10.255	1594	125	7006	7875	−896	−927
20	4660	9.7516	15.9400	5394	41.45	8.215	1386	153	6933	7891	−958	

[a] Average of three determinations.

[b] Calculated on the basis of 3300 gm. of water-free soil.

[c] Found by analyzing three 2-gm. samples and calculating from the weight of tops.

[d] Found by analyzing the entire root system.

[e] Based on analysis made at the close of the soybean series (data in table 10).

[f] The additions of nitrogen as clover tops made on these soils previous to the growth of soybeans are given as pounds of nitrogen added per 2,000,000 pounds of the mixture of soil and clover tops.

of nitrogen in 3300 gm. of soil was calculated from the preceding analyses. The soil which had been stored and used in this series was analyzed and found to contain the same amount of nitrogen as at the beginning of the soybean series, or 0.03136 per cent of nitrogen on the water-free basis, which gave 1034 mgm. of nitrogen per pot. The crop weights and the amounts of nitrogen in the crop are given separately as tops and roots.

Following the tables is a graphical representation of the nitrogen balance in the series (fig. 2).

In the series treated with sodium nitrate the amounts of total nitrogen present at the close were larger in every pot than those present at the outset.

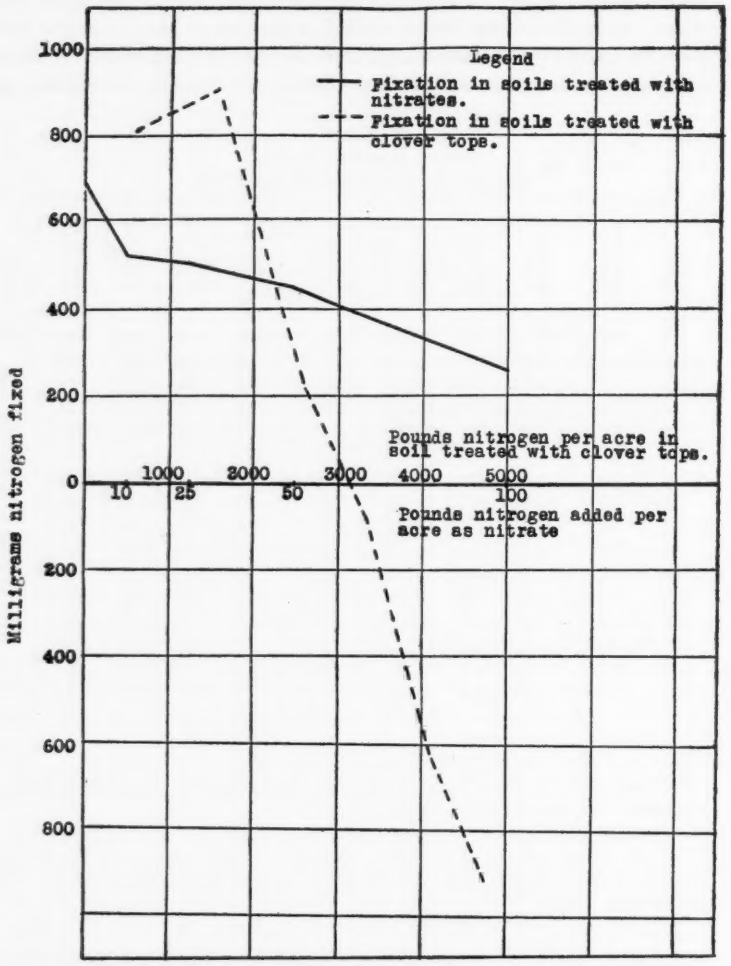

FIG. 2. GRAPHS SHOWING NITROGEN FIXATION BY COWPEAS ON SOILS TREATED WITH NITRATES, AND WITH CLOVER TOPS

This indicates decidedly that nitrogen was fixed from the atmosphere. The amounts so obtained were very significant, and in every case far above the limits of variation in analytical determinations. The average nitrogen in-

crease, or nitrogen fixation, from duplicate pots shows a gradual decrease with increase in nitrate nitrogen added to the soil. The largest fixation appeared in the pots whose soil received no nitrogen additions and was reduced about the same in the two soils treated at the rate of 10 and 20 pounds of nitrate nitrogen per 2,000,000 pounds of soil. On the soil treated with the equivalent of 100 pounds of nitrate nitrogen the fixation was about 38 per cent of that where no treatment was applied. The general decrease in fixation with increased treatment would lead one to believe that one or two more increments beyond the application of 100 pounds would have prohibited fixation completely. The table indicates that increasing amounts of nitrate nitrogen in the soil reduce nitrogen fixation by cowpeas, and may perhaps prohibit it, but the amounts required for significant reduction of this process are far greater than ever occur in a soil or are ever applied.

The series treated with organic matter and previously used for growing soybeans had some pots which failed to show any increase in total nitrogen at the close over that at the outset. Significant increases were obtained for the check pots and those originally treated with the equivalent of 1000 and 2000 pounds of nitrogen per acre, but for the rest of the treatments a negative fixation, or loss, took place. This was greater with larger applications of nitrogen. It is highly probable that the organic matter put on the soil in such heavy applications one season previously was still undergoing decomposition rapidly enough to lose nitrogen as ammonia or as gaseous nitrogen. Decomposition had not yet gone far enough to change all the organic matter of such heavy applications into a more stable form, from which no losses could take place. Such losses from the total nitrogen present in soil and crop at the close, as compared with that present in soil and seed at the beginning, do not prove that the plant failed to draw on the atmosphere for some of its nitrogen supply. Nodules were plentiful in the soils receiving the heaviest applications of organic matter and some use may well have been made of gaseous nitrogen. Any fixation that could have taken place was offset by the losses from the soil, and could not be detected in this method of determination.

Considering the averages for duplicate pots, the amounts fixed in the check pots 1 and 2, which had previously grown the uninoculated soybeans, were larger than that fixed by pots 3 and 4, on which the soybeans were inoculated. This difference between the two sets of check pots resulted from a larger amount of nitrogen being present in both the crop and the soil in the first two pots. The reverse was true, however, of the total crop weights, largely because of greater root development in pots 3 and 4. In pots 11 and 12 the average fixation was the largest in the series. This would indicate that the treatment with organic nitrogen corresponding to 1000 pounds per acre was beneficial to nitrogen fixation by cowpeas in a soil so low in this element as this soil was. This statement cannot be made, however, for all the higher applications. A decided increase was shown by the pots receiving 2000 pounds of nitrogen per acre, but those receiving more showed a loss rather than a gain.

Evidently the lower applications do not prohibit nitrogen fixation, but whether the larger ones prohibit the process cannot be said from the preceding data. The losses coming doubtlessly from the decaying organic matter in the soil more than balance any nitrogen from the air added to the plant. In terms of total nitrogen in the soil rather than nitrogen added, those pots showed nitrogen fixation whose soils contained the equivalent of approximately 625, 1625 and 2600 pounds of nitrogen per 2,000,000 pounds. From this it may be safely said that a soil with these amounts of nitrogen does not prohibit the symbiotic nitrogen fixation even when 2000 pounds of nitrogen were in a readily decomposable form. The soils with higher nitrogen content failed to show similar results because the large amount of nitrogen applied to the soil was partly lost in a volatile form.

Series 3 (Cowpeas)

The third crop series was very similar to the second, with a few modifications. It included one series of pots with soils treated with nitrates and another series with soils treated some time previously with organic matter in the form of clover tops.

For treatment with nitrates some soil saved by careful storage from the first crop series of soybeans was used. Sodium nitrate was added in solution to increase the nitrogen content at increments equivalent to 50, 100, 150, 200 and 250 pounds of nitrogen per 2,000,000 pounds of soil. Two additional pots receiving the equivalent of 50 pounds of nitrate nitrogen were included, but this was added in three applications at intervals extending over the greater part of the growing period. Three kilograms of soil were used per pot.

For soils whose nitrogen was increased by organic matter, those pots used in both preceding crop series were used again without change, save that the amounts of soil were less, and only 2800 gm. of water-free soil were weighed into each pot. The nitrogen content of these soils did not increase in constant amounts for the series, but corresponded very closely to amounts equivalent to 625, 1625, 2600, 3250, 4025 and 4775 pounds of nitrogen per 2,000,000 pounds of soil. These soils had been made up originally with enough clover tops to give a series whose nitrogen content increased by units of 1000 pounds, but the losses during the two preceding crop series reduced it to the above figures. It was thought advisable to use these soils again since their nitrogen content was accurately known, and the growth of two crops allowed sufficient time to permit decomposition of the organic matter to have gone far enough to prohibit further losses in volatile nitrogen.

The soils were treated with calcium carbonate at the rate of 2 tons per 2,000,000 pounds of soil. Phosphorus, potassium and other mineral plant-food elements were applied in solution when the dry soil was first moistened and then at intervals during the crop growth. All soils were treated similarly in this respect. Attempts were made to keep the moisture content of the soil

at optimum by weighing the pots, but this method was inaccurate when the plants became larger, so that water was applied at intervals to keep the moisture content near what seemed optimum.

Cowpeas were grown as the crop. Six seeds weighing in total an average of 1.109 gm. and containing 38 mgm. of nitrogen were planted in each pot. As the seedlings were well started, one was removed to leave five vigorous plants.

The growth of the crop was good. No difficulty was experienced in germination, and though the early growth was somewhat slow, the remainder of the growing season found the plants doing well. Differences due to treatment were manifested early. With increased amounts of nitrate applied the plants were taller, of a deeper color and the leaves more fleshy, but these marked differences disappeared later. The plants on soils treated with organic matter were larger and of deeper color as the nitrogen content of the soil increased. These variations became less prominent and were completely lost before the close of the experiment. The total plant growth was similar to that in the preceding crop series, but with less variations. Plate 4 illustrates the crop growth of the two series just before harvest, which was about 22 weeks after planting.

Shortly before the crops were harvested, several blossoms were formed but were removed to keep these few plants from setting seed. All blossoms and fallen leaves were collected and saved for analysis. The harvested plants were dried in the greenhouse, and later weighed and thoroughly ground for analysis.

The roots were carefully removed, examined for nodules and thoroughly dried. No great differences in nodule production were evident as correlated with the treatment. There were no significant differences shown by the nodules in the soils treated with nitrates. Nodules were numerous in all pots and especially so in those receiving the equivalent of 250 pounds of nitrogen per 2,000,000 pounds of soil. There was no evidence in either of the duplicate pots that the application of such large amounts of nitrate interfered with nodule production. This application was equivalent to 12.5 mgm. of nitrogen per 100 gm. of soil and its failure to suppress nodule growth agrees well with the results of Fred and Graul (17) who found that 10 mgm. of nitrate nitrogen per 100 gm. of Miami silt loam were required before nodules of alfalfa and crimson clover were decreased by such treatment. Evidently the application in the yellow silt loam used in this case was still less than was required for serious effect on nodule production.

Nodules were present in all pots treated with organic matter and gave indications of being larger and better distributed within those soils receiving the heaviest applications This may have been due to the better physical condition and greater aeration caused by such treatment. These results are not in accord with those found by Frank (14) for red clover, on which the nodules were fewer and smaller in the soil rich in organic matter.

At the close of this series, determinations of nitrate nitrogen were made on all the soils. Those treated with nitrate failed to show any nitrogen of this form present in the soil. This result is quite different from that found for soybeans, in which case an application of 258 mgm. per pot left much nitrate in the soil. The total nitrogen in the crop of soybeans was only 150 mgm. With the profuse growth of cowpeas in this series the application of 390 mgm. of nitrogen as nitrate was far below the nitrogen in the crop and might well be expected to be removed on account of its solubility. The removal was so complete that no more than a trace of nitrate was found in any of the soils.

TABLE 15

Nitrogen balance—Cowpeas II

Pot series 1, soil treated with nitrates

POT	NITRATE NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	WEIGHT OF CROP		NITROGEN IN THE CROP		NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
					Tops	Roots	Tops	Roots				
	mgm.	*gm.*	*mgm.*	*mgm.*	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1b	None	9.7363[a]	3.7909[a]	1168[b]	32.30	10.230	778[c]	153	2099	965[d]	1134	1212
2b	None	9.7455	3.8321	1179	36.35	11.740	909	168	2256	965	1291	
3b	75	9.7396	3.8543	1187	34.53	8.527	884	146	2217	1040	1177	1125
4b	75	9.7428	3.7909	1167	31.55	9.196	800	147	2114	1040	1074	
5b	150	9.7419	3.7223	1146	33.88	10.374	828	177	2151	1115	1036	1248
6b	150	9.7414	4.1902	1290	41.23	14.539	1109	177[e]	2576	1115	1461	
7b	225	9.7477	3.7863	1165	36.38	12.709	904	196	2265	1190	1075	1185
8b	225	9.7547	4.0892	1257	38.03	9.569	1064	165	2486	1190	1296	
9b	315	9.7486	3.9412	1212	37.08	10.756	968	175	2355	1280	1075	1092
10b	315	9.7535	4.1229	1268	36.78	12.215	929	192	2389	1280	1109	
11b	390	9.7453	3.9108	1203	38.53	9.831	1130	159	2492	1355	1137	1187
12b	390	9.7444	4.0892	1258	37.28	9.509	1138	197	2593	1355	1238	
13b	75[f]	9.7354	3.8368	1182	22.83	11.868	559	179	1920	1040	880	935
14b	75	9.7447	3.9850	1226	27.41	10.411	651	153	2030	1040	990	

[a] Average of three determinations.

[b] Calculated on the basis of 3000 gm. of water-free soil.

[c] Found by analyzing three 2-gm. samples and calculating from the weight of tops.

[d] Calculated on the basis of 3000 gm. of water-free soil with 0.0311 per cent of nitrogen, or 933 mgm. of nitrogen, and five cowpea seeds with 32 mgm. of nitrogen.

[e] Part of roots lost, and duplicate pot was used for data.

[f] Applied as three applications at intervals during the growth of the crop.

For the organic matter series about the same conditions obtained. No significant amounts of nitrates were present in any pots and only traces were found in those which had originally received the equivalent of 5000 pounds of nitrogen per acre as clover tops. Evidently the organic matter had undergone sufficient changes so that it no longer contained volatile organic compounds which interfered with the reduction method employed for determining nitrates. Nitrification may have been going on but not at sufficient rates to

permit measurable accumulations above the consumption by the plants. The rapid growth of the plants might well have removed all the nitrates formed by rapid nitrification processes. Since the determinations failed to show nitrates present, no tabulations of data are submitted.

To determine the total nitrogen in the crop and soil at the close, the procedure of the previous series was followed. All possible precaution was exercised to eliminate errors and maintain uniform results throughout. Tables 15 and 16 give the data of this series, showing the increase in total nitrogen at the close over that present at the beginning.

TABLE 16

Nitrogen balance—Cowpeas II

Pot series 2, soil treated with clover tops

POT	NITROGEN ADDED	SAMPLE WATER-FREE SOIL	NITROGEN IN SAMPLE	NITROGEN IN POT AT CLOSE	WEIGHT OF CROP		NITROGEN IN THE CROP		NITROGEN AT CLOSE (SOIL + CROP)	NITROGEN AT OUTSET (SOIL + SEED)	INCREASE, OR FIXATION	AVERAGE
					Tops	Roots	Tops	Roots				
	lbs.	*gm.*	*mgm.*	*mgm.*	*gm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	None	9.7280[a]	4.1271[a]	1187[b]	34.58	6.886	901[c]	136[d]	2224	1049[e]	1175	1138
2	None	9.7293	4.0034	1152	32.40	10.846	810	169	2131	1030	1101	
3	None	9.7392	3.7909	1089	27.20	9.466	619	162	1870	982	888	873
4	None	9.7321	3.7020	1064	25.00	8.044	607	148	1819	961	858	
11	995[f]	9.7427	7.2487	2083	35.00	6.378	1037	137	3257	1995	1262	1292
12	995	9.7498	7.4031	2126	39.55	5.951	1127	131	3384	2062	1322	
13	1960	9.7454	9.7580	2803	36.55	7.379	1160	145	4108	2786	1322	1295
14	1960	9.7398	9.7739	2809	39.40	6.727	1125	122	4056	2788	1268	
15	2890	9.7481	11.9310	3427	36.93	6.481	1039	124	4590	3344	1246	1261
16	2890	9.7576	11.8676	3405	33.93	6.014	1152	109	4666	3390	1276	
17	3790	9.7543	14.1009	4047	35.75	6.666	1012	117	5176	3946	1230	1215
18	3790	9.7608	13.3840	3839	38.26	8.482	1130	131	5100	3900	1200	
19	4660	9.7597	15.8616	4550	32.61	9.492	927	132	5629	4517	1112	1155
20	4660	9.7553	15.7601	4523	39.28	8.595	1136	148	5807	4609	1198	

[a] Average of three determinations.

[b] Calculated on the basis of 2800 gm. of water-free soil.

[c] Found by analyzing three 2-gm. samples and calculated from weight of tops.

[d] Found by analyzing entire root system.

[e] Based on analyses made at the close of the preceding cowpea series. Data in table 14 figured for 2800 gm. of water-free soil and five cowpea seeds with 32 mgm. of nitrogen.

[f] The additions of nitrogen as clover tops made on these soils previous to the growth of soybeans are given as pounds of nitrogen added per 2,000,000 pounds of the mixture of soil and clover tops.

The above data show most decidedly that the addition of nitrates in even larger amounts than were used in the previous series does not prohibit an increase of nitrogen fixation. The use of this form of nitrogen in the previous test with cowpeas gave a gradual decrease of fixation as the application of nitrate increased. In that series nitrate equivalent to 100 pounds per 2,000,000

pounds of soil permitted only 38 per cent as much fixation as no treatment, hence it suggested that double the application would prohibit fixation completely. In this series the applications were more than doubled and increased to as much as 250 pounds of nitrogen per 2,000,000 pounds of soil, yet no serious hindrance was given to nitrogen fixation. The outstanding characteristic of the data is the lack of variations in nitrogen fixed. The average figures for duplicate pots show a fixation of approximately 1200 mgm. for all pots except the two receiving 315 mgm. of nitrate nitrogen at the outset, and the two receiving 75 mgm. in three successive applications. Those pots with 390 mgm. of added nitrogen gave a fixation of 1187 mgm., corresponding closely to the fixation of 1124 and 1212 mgm. in the check pots. The data indicate that these amounts of nitrate nitrogen used in this series had no significant effect either favorable or unfavorable, and the highest treatment gave no significant variation from the general uniformity of the series.

In pots 13b and 14b receiving three applications of 25 mgm. each or a total equivalent to 50 pounds of nitrogen per 2,000,000 pounds of soil, the fixation was the lowest in the series. The crops on these pots were the smallest of all pots, as is shown by the weights. The roots were about as heavy in these pots as any in the series, but the tops weighed far less than those of any other treatments. The same relations held for the total nitrogen in the tops and roots of these two pots. That of the tops was less, and that in the roots was about the equal of those of the other treatments of the series.

The fact that the fixation in pots 13b and 14b was not equal to that of 3b and 4b might lead one to believe that the application of 50 pounds of nitrate at the time the seed was planted gave more fixation than this same amount of nitrogen distributed over three applications during the plants' growth. The soluble nitrogen applied early may have enabled the plant to establish itself better at the outset and fix more nitrogen than when the same amount of soluble nitrogen was put into the soil at intervals. Pots 3b and 4b were treated exactly the same as pots 13b and 14b, save that the latter two did not receive all the nitrogen at the beginning, and such conclusions might be drawn from the comparison alone. When both these treatments are compared with the check, however, the interpretation must be different. The fixation when all the nitrates were applied at the outset was no larger than that of the check receiving no treatment, and was less than that of the check when applied at intervals during the plants' growth. On this basis the application of all nitrogen at the beginning showed no influence on the fixation, while the application at intervals kept it down..

The series with nitrates gives large amounts of nitrogen fixation for all pots, but little variation caused by treatments. It would indicate that nitrates used in amounts as high as 1500 pounds per acre do not exert any effect, unless perhaps when the applications are made at intervals during the plants' growth rather than at the beginning.

The second set of pots, whose total nitrogen content of the soil increased by approximately 1000 pounds per acre, showed results quite different from those it gave in its previous crop of cowpeas. Instead of losses of nitrogen, there were marked gains in all pots, indicating that the decaying organic matter had no longer lost nitrogen and that the nitrogen taken from the air appeared as increase in the total determinations at the close of the series. Check pots 3 and 4 whose nitrogen in the soil and crop was lower than that in check pots 1 and 2 in the previous crop of cowpeas, showed similar results again. The differences in the soil nitrogen of these two sets of checks amounted to more than 100 mgm. and in the crop they were even greater, thus giving a difference of over 250 mgm. in the amounts of nitrogen fixed by the two sets of checks. The use of one or the other set of checks as a basis for comparison will influence the interpretation of the results of the treatments, but only in degree rather than in kind, since all the treatments gave results above even the highest of the checks. The results from the four check pots were used as a basis in calculating the increase, or nitrogen fixation, as influenced by treatment.

By using the average of the four check pots as a basis for comparison all the treatments with organic matter increased the fixation of nitrogen. For those pots receiving the heaviest applications the increase is not as large as in the others, but all pots below this addition of 5000 pounds of nitrogen per acre gave a significant increase in fixation over even the highest of the check pots. This indicates that the addition of the organic matter increased the fixation, rather than hindered it, and that the soils richer in total nitrogen permitted the plant to draw on the atmospheric supply of this element as well as on that in soil. These facts are not in accord with the general statements often made, that the plant fails to draw on the nitrogen of the atmosphere until forced to do so by the deficiency in the soil. Here the results were the very reverse and the addition of organic nitrogen enabled the plants to take more nitrogen from the air as a result of such treatment. Such benefit was suggested even in the two previous crops, where the addition of 1000 pounds of nitrogen as organic matter gave in every case greater fixation than in soils not so treated. According to these data 1000 pounds of nitrogen added as organic matter increased nitrogen fixation as much as the larger applications used in the series and the fixation decreased slightly with increased additions, but this decrease was so small as to be insignificant. Even if this decrease were larger there would be no cause for alarm since the application of 1000 pounds of nitrogen as organic matter is equivalent to approximately 25 tons of clover tops and is far greater than is ever applied to soils for fertilizer uses, and such amounts of readily decomposable nitrogenous materials seldom or never occur in common soils.

The graphic representation of the data in figure 3 shows that cowpeas growing on soils treated with nitrates, or rich in organic matter may utilize atmospheric nitrogen even when the amounts of these forms of nitrogen are very large. They show further, that neither the nitrate treatment nor the soil's

high total nitrogen content affect nitrogen fixation detrimentally, but rather, that the treatment with organic matter favors the process by increasing the amount of atmospheric nitrogen taken by this legume.

The total nitrogen content in the soil of the one series was equivalent to 775, 1500, 2000, 2440, 2800 and 3240 pounds in 2,000,000 pounds, while the

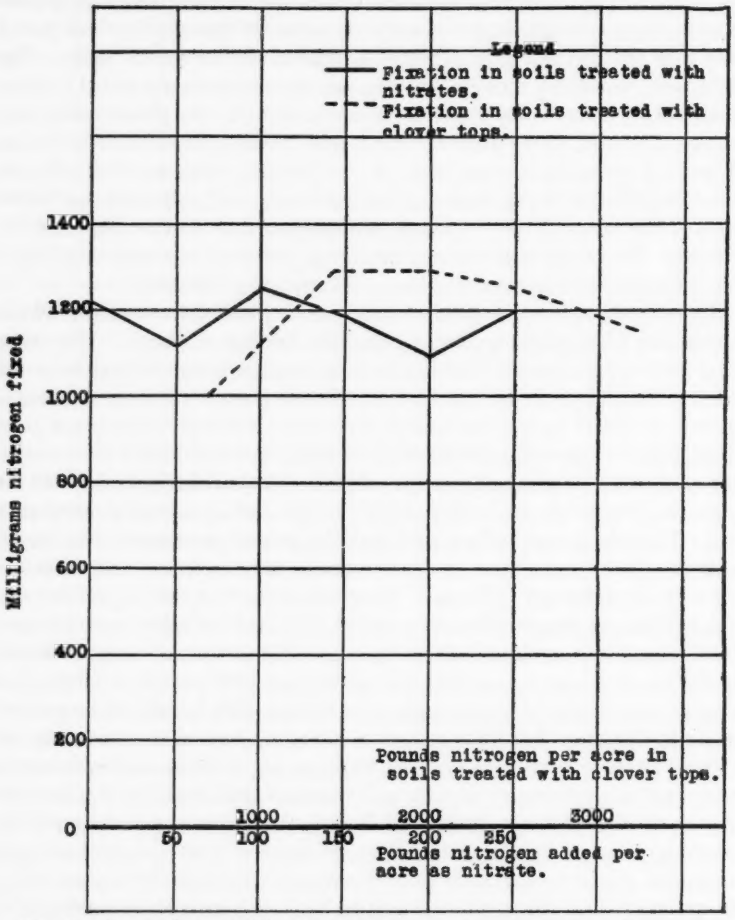

FIG. 3. GRAPHS SHOWING NITROGEN FIXATION BY COWPEAS ON SOILS TREATED WITH NITRATES, AND WITH CLOVER TOPS

fixations for these were 1005, 1292, 1295, 1261, 1215 and 1155 mgm. per pot, respectively. The increases in fixation caused by the organic matter over that of the check are 287, 290, 256, 210 and 150 mgm. with the increasing nitrogen content of the soil. Accordingly, the soils richer in nitrogen gave somewhat

less fixation than the soils poorer in this respect, but these differences are not significant when compared with the total nitrogen fixed. It may be possible that the higher treatments still lost some nitrogen from the soil during this time, and if proper corrections could be made they might show the same fixation throughout the series.

Nitrogen changes in soils during the growth of legumes

Since the soils used in this experiment were carefully analyzed for each crop grown on them, they offer an excellent opportunity for study of the changes in the nitrogen taking place within the soils themselves.

The soils in pots 1 to 4 and 11 to 20 were used during the entire series of three successive crops. The roots were removed as completely as possible to prevent addition of organic matter in this form. As these soils received applications of larger amounts of clover tops at the outset, they offer an interesting study of the changes in nitrogen content while this organic matter was decaying, and while three successive crops of legumes were growing. The following data are tabulated to give the changes in the nitrogen content of these soils as shown at the close of each crop. In the treated soils, from which there were losses of nitrogen, some estimate of these losses can be made; while in the untreated soil some measure may be taken of the influence of the legume plant on the nitrogen content of the soil itself. The figures were obtained from the analyses of the soil and organic matter at the beginning and from that of the soil alone at the close of each crop series. For the sake of uniformity the data are tabulated as pounds per acre of 2,000,000 pounds of soil. The data are given in table 17.

For the soils treated with organic matter, the losses rather than gains in nitrogen are the chief characteristics. During the growth of the soybeans no losses took place in the soils treated with less than 2890 pounds of nitrogen per acre. For this application the average loss was 242 pounds. For the two higher treatments the average losses were 382 and 510 pounds.

During the growth of the second crop, cowpeas, there were losses in all pots, and all were greater than occurred in these same soils during the first crop. The lowest two treatments, which had lost no nitrogen previously, showed decreases equivalent to 205 and 614 pounds per acre, while in those pots with larger applications of organic matter, the nitrogen losses were 892, 1254 and 1539 pounds. In terms of the original amounts of nitrogen applied these losses correspond to 20.6, 30.3, 33.0, and 33.0 per cent, respectively. Evidently the most rapid decomposition and heaviest losses did not take place until the time of the second-crop growth, which closed 201 days after the soils were treated. Drying and grinding the soil between the two crops may have hastened decomposition.

For the period of time in which the third crop was grown the data show results different from those for the two preceding periods. The treated soils no

longer gave losses of nitrogen, but in all cases gave gains for this element. The largest average gain was 77 pounds in the pots receiving 995 pounds of nitrogen per acre, but in the other pots the gains decreased with increased treatments, though in no regular order. These increases in the soil nitrogen are not large, but big enough to be considered as increases as measured by accurate analytical determinations varying less than 0.2 mgm. of nitrogen in 10-gm. samples of soil in triplicate determinations. This range in difference corresponds to 40 pounds per acre, and the error becomes less when three samples are taken. Even though these increases were not considered, there is no small importance in the fact that these soils produced such heavy crops

TABLE 17

Changes during the growth of three crops of legumes in a soil treated with organic matter

All figures given in pounds per 2,000,000 pounds of soil

POT	CLOVER TOPS ADDED	NITROGEN ADDED	NITROGEN IN THE SOIL						
			At the beginning	After first crop (soybeans)	Increase	After second crop (cowpeas)	Increase	After third crop (cowpeas)	Increase
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	None	None	625	583	−18	726	113	847	115
2	None	None	625	630		713		822	
3	None	None	625	633	−2	678	48	778	98
4	None	None	625	613		664		760	
11	37,766	995	1620	1600	11	1402	−205	1488	77
12	37,766	995	1620	1663		1450		1518	
13	75,532	1960	2585	2550	−2	1967	−615	2002	35
14	75,532	1960	2585	2615		1968		2006	
15	113,298	2890	3515	3185	−242	2365	−892	2448	60
16	113,298	2890	3515	3360		2395		2432	
17	151,064	3790	4415	4110	−382	2795	−1254	2890	37
18	151,064	3790	4415	3955		2762		2742	
19	188,830	4660	5285	4770	−510	3203	−1538	3250	4
20	188,830	4660	5285	4780		3269		3230	

of legumes and yet suffered no loss in nitrogen. In some cases the nitrogen in the total crop was over half as much as that originally in the soil. Had the crop taken its nitrogen from the soil, such amounts removed could easily have been measured by ordinary analytical methods.

The greatest increases in nitrogen of the soil occurred in the four check pots which had been left untreated. During the growth of the soybeans these soils showed no significant change, save a very slight loss. During the next crop, which was cowpeas, they showed decided gains in nitrogen, and again gave similar results for the last crop of this same legume. The soils in all four pots increased in nitrogen by growing the cowpeas. It cannot be specifically said whether this was due to a direct change in the original soil material itself, or to the addition of small roots and nodules that remained in spite of the careful

attempt to remove them; but it seems most probable that the latter reason was sufficient to give the increases recorded. With such gains in soil nitrogen when the roots are removed, surely the incorporation of the roots themselves would give a decided increase. It has been said for some legumes (25, p. 218) that the addition of merely the roots does not increase the nitrogen content of the soil, but the above data indicate that the 625 pounds of nitrogen originally present in this soil increased when the roots were removed as carefully as possible, and would have increased much more if the roots had been included. The same may be said of the richer soils during the last crop of cowpeas, all of which showed some gains in nitrogen content when the roots were removed. Had the roots been left in the soil its nitrogen content would have been raised decidedly, even where the total nitrogen of the soil was already equal to 3200 pounds per acre. None of the soils in the last crop series, whether high or low in nitrogen, lost in this element as the result of the removal of the entire crop of roots and tops of the plants. All would have been enriched in this respect had only the roots been added.

Distribution of nitrogen in roots and tops of cowpeas

It has been known that the portions of the total plant substance which are in the tops and in the roots are not constant for the different kinds of plants, nor are they always constant for the same kind of plant (24, 40, 49). Similar statements may be made concerning the distribution of the constituent elements of the plant tissue, especially the nitrogen. The data of this experiment were taken in the hope of studying the distribution of the nitrogen in the tops and roots, as well as the weight of these plant parts as influenced by the nitrogen content of the soil. The plants were harvested by cutting the stems just level with the soil surface, and considering as tops that part above the soil level, and as roots all parts below it. Only the two cowpea crops were treated in this manner and the data are given for this legume grown in a soil with varying amounts of nitrates and in a soil containing varying amounts of organic nitrogen. The data were taken from the previous table and are given as averages of the duplicate pots for each treatment (tables 18 and 19). The distributions of the weights and nitrogen in the tops and roots are expressed as per cent of the total.

According to the data, the addition of nitrate nitrogen tends to increase the weight of the plant top faster than that of the roots. In the first cowpea crop, the percentage of the total plant weight in the tops increased gradually while that in the roots decreased as the amount of nitrate nitrogen added became larger. In the second crop there was also an increase, but not such a marked one. The entire second series showed a higher percentage of the total plant weight in the top than was shown in the previous series. Evidently some other factor was coupled with the nitrogen in the soil to influence the distribution of the weights in tops and roots. This may well be expected since the

TABLE 18

Distribution of nitrogen in the roots and tops of cowpeas

Soil treated with nitrates

NITROGEN ADDED PER POT	PART OF PLANT	WEIGHT	PER CENT OF TOTAL	TOTAL NITROGEN	PER CENT OF TOTAL
		First crop			
mgm.		*gm.*		*mgm.*	
None	Tops	15.25	62.51	416	72.40
	Roots	9.145	37.48	158	27.52
16.5	Tops	15.60	66.63	373	71.45
	Roots	7.810	33.36	149	28.54
41.0	Tops	16.05	68.26	368	68.91
	Roots	7.463	31.73	166	31.08
82.0	Tops	16.40	67.09	424	71.99
	Roots	8.043	32.90	165	28.00
157.0	Tops	15.125	69.86	366	73.34
	Roots	6.704	30.71	131	26.25
		Second crop			
None	Tops	34.32	75.75	843	84.04
	Roots	10.985	24.24	160	15.95
75.0	Tops	33.04	78.85	841	85.20
	Roots	8.861	21.14	146	14.79
150.0	Tops	37.55	75.09	968	84.54
	Roots	12.456	24.90	177	15.45
225.0	Tops	37.20	76.95	984	84.53
	Roots	11.139	23.04	180	15.46
315.0	Tops	36.93	76.27	948	83.81
	Roots	11.485	23.72	183	16.18
390.0	Tops	37.90	79.61	1134	86.43
	Roots	9.705	20.38	178	13.56
75.0	Tops	25.12	69.27	605	78.46
	Roots	11.139	30.72	166	21.53

TABLE 19

Distribution of nitrogen in the roots and tops of cowpeas

Soil treated with clover tops

NITROGEN IN SOIL	PART OF PLANT	WEIGHT	PER CENT OF TOTAL	TOTAL NITROGEN	PER CENT OF TOTAL
		First crop			
lbs.[a]		*gm.*		*mgm.*	
None	Tops	20.67	76.05	581	81.71
	Roots	6.508	23.94	130	18.28
None	Tops	20.32	70.07	503	75.07
	Roots	8.676	29.92	167	24.92
995	Tops	34.92	76.68	1049	83.78
	Roots	10.615	23.31	203	16.21
1960	Tops	36.37	75.77	1051	84.21
	Roots	11.630	24.22	197	15.78
2890	Tops	37.82	75.73	1170	84.47
	Roots	12.114	24.26	215	15.52
3790	Tops	40.45	81.53	1273	88.15
	Roots	9.161	18.46	171	11.84
4660	Tops	43.57	82.51	1490	91.46
	Roots	9.235	17.48	139	8 53
		Second crop			
719	Tops	33.49	79.06	855	84.90
	Roots	8.866	20.91	152	15.09
621	Tops	26.10	74.88	613	79.81
	Roots	8.755	25.11	155	20.18
1426	Tops	37.77	85.96	1082	88.98
	Roots	6.164	14.03	134	11.01
1967	Tops	37.97	84.33	1142	89.56
	Roots	7.053	15.66	133	10.43
2381	Tops	35.43	85.01	1095	90.42
	Roots	6.247	14.98	116	9.57
2778	Tops	37.00	83.00	1071	89.62
	Roots	7.574	16.99	124	10.37
3236	Tops	35.94	79.89	1031	87.29
	Roots	9.043	20.18	150	12.70

[a] Pounds in 2,000,000 pounds of soil.

crops were grown at different seasons. Nevertheless, the nitrogen in this readily soluble form was of influence, and increased the plant growth above the ground more than it did the development of the root system.

In the soils with varying amounts of organic nitrogen, the higher nitrogen content of the soil gave a greater portion of the plant weight in the tops, but there was no regular increase in this proportion with the regular increase in the soil nitrogen. This failed to show the uniformity of increase shown by the soils treated with nitrates, but yet indicated that as the soils were richer in nitrogen, the weight of the plant above ground increased faster than the weight of the roots.

Considering the distribution of the total nitrogen of the plant, the effects of the nitrates of the soil toward increasing the portion of this in the tops are not as marked as for increasing the weight of the tops. The data of both series fail to show any regular influence, by the nitrates, on the percentage of the total plant nitrogen found in the roots or the tops. The effect of the organic nitrogen in the soil was shown as a more rapid increase in the nitrogen of the tops, than that of the roots. In the first crop this effect was very decided, giving 10 per cent more of the plant's total nitrogen in the tops for the soil with high nitrogen content than for the soil to which no organic nitrogen had been added. In the second crop, all the soils richer in nitrogen than the untreated soil had a bigger portion of the plant's nitrogen in the tops, but there was no close correlation between the soil treatment and the disturbance in the distribution of the total nitrogen in the plant tops and roots. Nevertheless, it is evident that the nitrogen in the part of the plant above ground increased with the treatment of both nitrates and organic matter.

Between the distribution of the nitrogen, and the fixation of this element, there is no correlation in this experiment. It cannot be said that the fixation is greater with increased production of that part above the ground, which is the relation that one might expect according to Peterman (41). Scarcely enough data were secured to warrant a specific statement on this question of relation of crop size to the amount of nitrogen fixation.

IV. SUMMARY

The experiment herein reported was a study of nitrogen fixation as influenced by the nitrogen content of the soil. Variations in the nitrogen of the soil were brought about by adding sodium nitrate, and by incorporating organic matter in the form of clover tops in a soil containing only 625 pounds of nitrogen per 2,000,000 pounds of soil. This gave variations in mineral nitrogen and organic nitrogen, respectively.

One crop of soybeans and two crops of cowpeas were grown. Some of the original soil was used each time for the treatment with nitrates, but the same soils were used throughout the three crops for the treatment with organic matter.

Nitrogen fixation is represented as the increase in the total nitrogen in the soil and crop at the close of the experiment, over that present in the soil and seed at the beginning.

The decaying organic matter occasioned some heavy losses in nitrogen from the soil. These losses reached the maximum during the second crop, and had apparently disappeared after 200 days. During the second crop, the highest losses of nitrogen from the soil were equivalent to 1538 pounds per 2,000,000 pounds of soil where the original application of clover tops was equivalent to 4660 pounds of nitrogen per acre. These losses from the soil with the heavy applications of organic matter made it impossible in a few instances to measure the nitrogen fixation. In those soils, however, to which were added nitrates and smaller quantities of organic matter, nitrogen fixation was easily measured and a decided amount was found.

The addition of the approximate equivalent of 1000 and 2000 pounds of nitrogen as organic matter per acre, corresponding to 18 and 36 tons of clover tops, respectively, gave nitrogen fixation for all three crops grown. For the last crop grown, during which time the soils no longer showed losses of nitrogen, there were decided gains, or nitrogen fixation, for all treatments with organic matter.

Nitrates did not prohibit nitrogen fixation. Indications in one series of cowpeas suggested that increasing amounts of nitrogen applied as sodium nitrate lessened the amount of fixation. A later series of the same crop with higher and wider ranges of the application of nitrates failed to show the same results, even when as much as 250 pounds of nitrogen was applied in this form. This corresponded to an application of more than 1500 pounds of sodium nitrate per acre.

The addition of sodium nitrate to the soil caused the plants to grow better at the beginning, but gave no increased nitrogen fixation for two series of cowpeas, save in one treatment of 150 pounds of nitrogen per acre, where the fixation was slightly greater than that of the check. Fixation in all other treatments with nitrates was less than that of the check.

After the soils treated with organic matter ceased to lose nitrogen by decomposition, they showed a large nitrogen fixation. The organic matter added caused this fixation to be larger than that in the soils not so treated. The increase in the nitrogen fixed as caused by the organic matter, was not proportional, however, to the amount of organic matter applied.

There were some variations in the amounts of nitrogen taken from the air by five plants of cowpeas in each pot. The maximum average fixation for duplicate pots was 1295 mgm. on a soil containing the equivalent of 2000 pounds of total nitrogen per acre. Amounts almost as large were obtained in soils treated with 150 pounds of nitrate nitrogen per acre.

Nodule production was not suppressed to an appreciable extent by any of the treatments of nitrate or organic matter.

The untreated soils which grew legumes for three successive crops showed gains in their nitrogen content as a result of these crop growths, even though all roots were removed from the soil as completely as possible. In the last crop of cowpeas grown, all soils from which the roots had been removed, became richer in nitrogen under this treatment, even though some of these soils contained more than 3000 pounds of nitrogen per acre before the crop was grown, and the crop itself contained more than one-fourth of this amount. This fact indicates that the growth of a legume may be large, but yet leave the nitrogen content of the soil nearly constant or even increase it, and that the addition of the plant roots will increase the soil's nitrogen content when there is no loss by leaching.

As the soil's content of total nitrogen or of nitrate nitrogen was higher, the yield of plant tissue became greater; and the greater part of this increase was in the tops of the plant rather than in the roots. With a higher nitrogen content of the soil, a bigger share of the plant's total nitrogen was also in the tops.

V. CONCLUSIONS

1. The results of this study indicate that nitrogen fixation will take place in a soil containing large amounts of nitrogen in the form of either nitrates or organic matter.

2. No injurious effects on nitrogen fixation were caused by nitrates in this experiment, and if such ever occur under conditions similar to those which obtained in this study, the application of nitrates must be many times larger than is ever applied in agricultural practice.

3. Nodules are produced when large amounts of organic nitrogen are present in the soil, and good legume growth results even when sufficient organic matter is present to give large losses of volatile nitrogen from the soils.

4. The addition of some organic matter may increase the amount of nitrogen fixed by cowpeas.

5. In soils containing varying amounts of total nitrogen, as much fixation of nitrogen by cowpeas may be expected in one with 3000 pounds of total nitrogen, as in one with lesser amounts. According to the data given, variations in the amount of total nitrogen in a soil failed to exert any varying influence on the amount of nitrogen fixed.

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PLATE 1

SOYBEANS ON SOIL TREATED WITH NITRATES, CLOVER TOPS, AND WITH CLOVER TOPS AND NITRATES

Yellow Silt Loam
Check
Yellow Silt Loam
Check
Clover Tops
Yellow Silt Loam
Check
Clover Tops
Yellow Silt Loam
Check
Clover Tops

PLATE 2

COWPEAS, AT 50 DAYS, ON SOIL TREATED WITH NITRATES (ABOVE) AND COWPEAS AS THE SECOND CROP ON SOIL TREATED WITH CLOVER TOPS (BELOW)

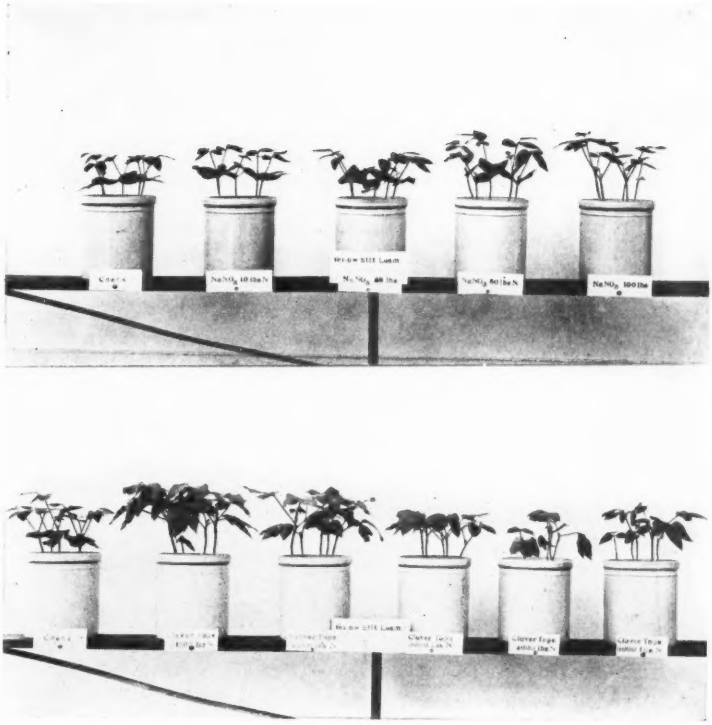
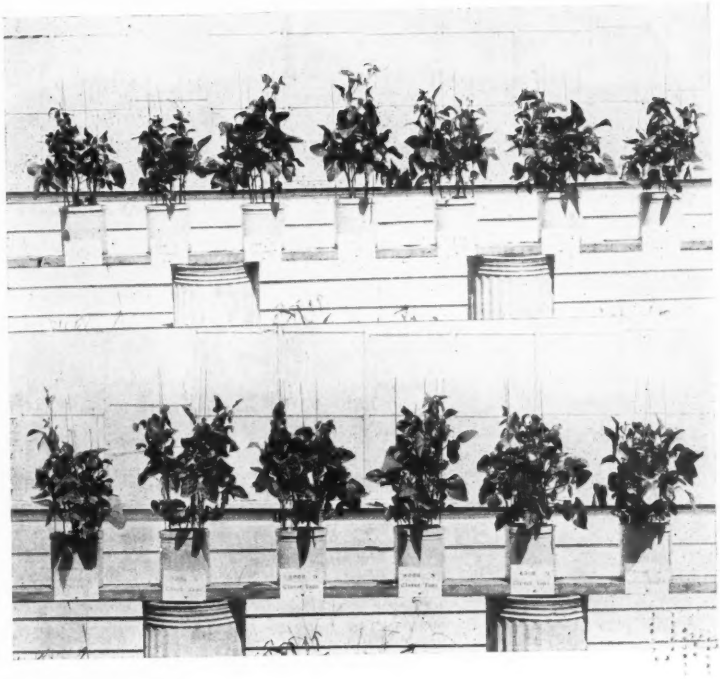


PLATE 3

COWPEAS, AT 75 DAYS, ON SOIL TREATED WITH NITRATES (ABOVE) AND COWPEAS AS THE SECOND CROP ON SOIL TREATED WITH CLOVER TOPS (BELOW)

PLATE 4

COWPEAS ON SOIL TREATED WITH NITRATES (ABOVE) AND COWPEAS AS THE THIRD CROP ON SOIL TREATED WITH CLOVER TOPS (BELOW)



THE POTENTIAL BIOCHEMICAL ACTIVITY OF THE SPORES OF SOIL BACTERIA[1]

J. R. NELLER

New Jersey Agricultural Experiment Station

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INTRODUCTION

The microörganic population of an arable soil must accommodate itself to great fluctuations in moisture, temperature, aeration and food supply. Obviously only those types survive from season to season which are able to live during the unfavorable periods. Applications of manure and of decaying organic matter will introduce non-spore-forming organisms which may be active while there is sufficient food and moisture, but tend to disappear during subsequent exposure to desiccation. Conn and Bright (3, p. 325) found that non-spore-formers were only occasionally isolated from a soil which had not been manured for some time, while the spore-formers were always present in fairly constant numbers.

Thus it seems evident that considerable of the work of liberating plant-food from plant residues and from dried organic matter previously added, is performed under optimum moisture conditions and by organisms which previously existed as resting bodies or spores: In order to measure the potential biochemical activity as well as to study the distribution of spores existing at any given time, laboratory tests were conducted with heated and unheated infusions of fresh soil. These samples were taken at intervals during 1916 and a part of 1917, and were obtained to a depth of $\frac{2}{3}$ meter on two distinctly different types of arable soil.

It is hard to determine the amount of heat which should be applied to an infusion as cosmopolitan as that of the soil in order to destroy the vegetative cells and as few as possible of the spores. Lipman (6, p. 230) used a temperature of 80°C. for 10 minutes and found that infusions thus heated and inoculated into sterile peptone solution produced nearly as much ammonia at the end of 4 days as the unheated infusions. Conn (2, p. 188) used a temperature of 75°C. for 15 to 20 minutes, and found that the numbers of spore-formers appearing on gelatine plates was nearly as great with the heated as with the unheated inoculum. He also obtained no increase in the numbers of spore-formers as a result of adding heavy applications of manure to a soil and concluded that sporulating bacteria of the soil multiply but slightly and exist for the most part as inactive spores.

[1] Technical Paper No. 2 of the New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

Hiss and Zinsser (4) state that heating to 55° or 60°C. for 10 minutes causes the death of most vegetative forms. · Lauback (5, p. 493) used a temperature of 90°C. for 15 minutes for his isolation of non-pathogenic bacterial spores. Meyer (7, p. 127) studied the thermal death-points of the spores of various pure cultures and found that the most sensitive could withstand a temperature of 80°C. for nearly 3 hours.

The infusions used in this work were heated to 85°C. for 10 minutes. This temperature undoubtedly destroyed some of the spores as well as the vegetative cells, but it also destroyed nearly all of the fungi and all of the actinomycetes and chromogenic bacteria. At least these organisms failed to appear on the plates inoculated with the heated infusions, but were numerous on those inoculated with unheated infusions.

METHODS

The soil samples were obtained to a depth of 64 cm. at intervals of about 2 months by means of a bacteriological soil sampler previously described (8, p. 109). With the aid of this sampler, it was possible to secure each of the five samples from a small area without affecting the soil from which the following samples were to be taken. The samples were placed in sterile fruit jars in the field and infusions were obtained from them the same day. The infusions were prepared by weighing 50 gm. of the fresh soil into Erlenmeyer flasks containing 150 cc. of sterile water. These were shaken vigorously for 5 minutes and allowed to settle, after which portions were pipetted off for the heated and unheated inoculations. The portions to be heated were pipetted into selected thin-walled test-tubes. By means of rubber bands these tubes were held in place against the sides of a wire basket which was then placed in a large dish of water heated to a temperature of 87°C. The insertion of the basket and tubes would bring the temperature down to 85°C., where it was maintained. A control test-tube containing a thermometer indicated when the infusions had reached a temperature of 85°C. The water surface around the outside of the test-tubes was always higher than that within and the basket was moved about sufficiently to keep the liquids within the tubes in circulation. At the end of the 10-minute period, the tubes were withdrawn and allowed to cool.

Dilutions were made from all infusions thus prepared for the determination of numbers for which Brown's egg albumin agar (1, p. 381) was used. Counts were made after incubating the plates at from 20° to 22°C. for 8 days.

For the ammonia and carbon-dioxide determinations, 250-cc. Erlenmeyer flasks containing 70 gm. of soil, 100 mgm. of nitrogen in dried blood and water equivalent to 50 per cent of the maximum capacity of the soil, were sterilized at a pressure of one atmosphere for 30 minutes. These flasks were inoculated with 5 cc. of soil infusion and incubated for 7 days at from 20° to 22°C. Uninoculated flasks were incubated in several cases, but gave no

ammonia accumulation over that originally present, nor carbon-dioxide production greater than the blank determinations on the apparatus. The ammonia was determined by the magnesium oxide method, and the carbon dioxide by a method previously described (9, p. 226).

THE RELATIVE NUMBERS AND DISTRIBUTION OF BACTERIA SPORES IN TWO TYPES OF SOIL AT VARIOUS DEPTHS AND SEASONS

Bacterial numbers were determined on all infusions of samples obtained for biochemical tests. These samples were obtained on August 8, November 28, January 9, April 3 and June 14, from tilled fields of Sassafras gravelly

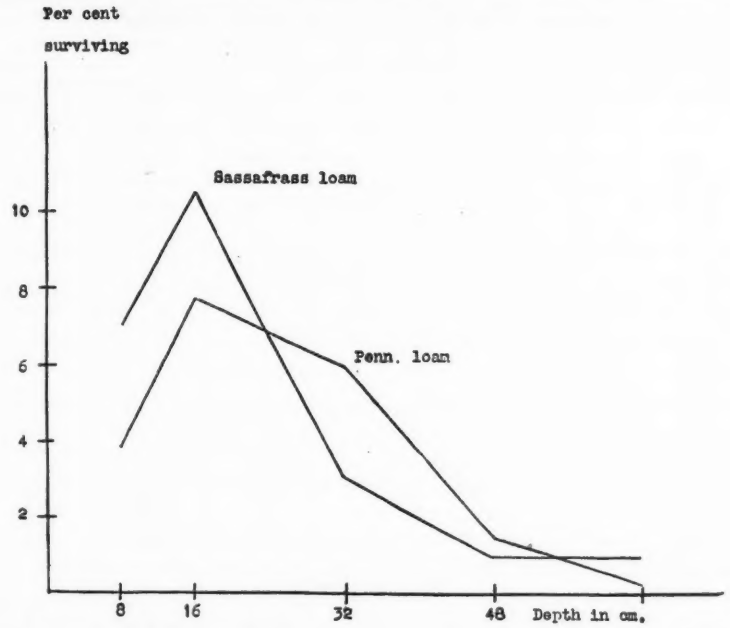

FIG. 1. CURVES SHOWING THE RELATION BETWEEN THE INCREASE IN DEPTH OF SOIL AND THE DECREASE IN BACTERIAL NUMBERS WHICH SURVIVED HEATING TO 85°C. FOR 10 MINUTES (Table 2)

loam and of Penn shale loam. No manure or fertilizer was added to these fields during the two growing seasons during which the samples were taken. These two soils are very unlike in texture, as may be seen from the moisture record in table 1. Unheated infusions of the finer-textured shale loam gave a much higher count for the surface layers, but not for the subsoil. That a relatively greater number survived the application of heat in the surface layers of the coarser-textured gravelly loam is evident from the percentage values in table 2.

In the lower layers of both types of soil, however, the numbers of spores fell off much more rapidly than the total numbers of organisms. This is evident for all of the sampling (table 2), which, when averaged, give 5.8 per cent and 8.7 per cent surviving for the first 16 cm. of surface soil, but only 2.7 per cent and 1.6 per cent for the first 32 cm. of the subsoil. This

TABLE 1

Bacterial numbers in heated and unheated infusions of Penn shale loam and of Sassafras gravelly loam

DEPTH OF SAMPLING	SASSAFRAS GRAVELLY LOAM			PENN SHALE LOAM		
	Number of bacteria		Water content of soil	Number of bacteria		Water content of soil
	Unheated	Heated		Unheated	Heated	
Sampling of August 8						
cm.	*millions per gm.*	*millions per gm.*	*per cent*	*millions per gm.*	*millions per gm.*	*per cent*
0– 8	1.38	0.110	2.0	11.00	0.150	18.5
9–16	3.47	0.120	2.5	5.50	0.310	17.8
17–32	3.33	0.090	12.9	1.80	0.100	20.5
33–48	1.43	0.006	6.4	0.29	0.008	25.0
49–64	0.98	0.001	10.0	0.05	0.0003	20.5
Sampling of November 28						
0– 8	5.20	0.370	16.3			
9–16	4.90	0.400	16.3			
17–32	2.00	0.053	12.3			
33–48	0.49	0.010	12.3			
Sampling of January 9						
0– 8	6.60	0.800	16.7	29.00	2.080	31.0
9–16	4.50	1.500	16.7	25.90	4.790	31.0
17–32	1.50	0.250	9.5	3.40	0.280	26.0
33–44	0.17	0.002	9.0	3.10	0.009	30.0
Sampling of April 3						
0– 8	7.90	0.350	13.6	6.30	0.270	25.0
9–16	4.80	0.140	23.0	8.20	0.310	25.5
17–32	1.40	0.030	11.5	1.80	9.100	25.0
33–48	0.73	0.002	10.5	0.33	0.003	24.0
49–64	0.09	0.002	11.0	0.03	None	24.5
Sampling of June 14						
9– 8	3.96	0.162	7.5	15.50	0.186	22.0
9–16	2.31	0.117	10.2	7.00	0.245	26.6
17–32	0.61	0.037	6.4	6.10	Lost	26.6
33–48	Lost	0.009	4.2	0.22	0.006	25.0
49–64	0.21	0.001	4.2	0.25	0.005	28.2

TABLE 2

The percentage of the bacterial numbers which survived heating to 85°C. for 10 minutes

DEPTH OF SAMPLE	PENN SHALE LOAM; SAMPLING OF					SASSAFRAS GRAVELLY LOAM; SAMPLING OF					
	January 9	April 3	June 14	August 8	Average	November 28	January 9	April 3	June 14	August 8	Average
cm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–8	7.2	4.5	1.2	1.4	3.8	6.1	12.0	4.0	4.1	8.0	6.8
9–16	18.5	3.8	3.5	4.6	7.8	8.1	33.0	3.0	5.1	3.5	10.5
17–32	8.5	5.5	Lost	5.6	6.5	2.7	1.6	2.1	6.0	2.7	3.1
33–48	0.2	1.0	0.3	2.8	1.1	2.0	1.0	0.3	Lost	0.4	0.9
49–64	None		0.2	0.6	0.21			2.0	0.5	0.1	0.9

great decrease (fig. 1) in the relative numbers of spores in the subsoil is possibly due to the fact that the subsoil flora does not undergo such extreme fluctuations in environmental conditions.

THE RELATIVE ACTIVITY OF HEATED AND UNHEATED INFUSIONS AS SHOWN BY THE AMMONIA ACCUMULATIONS

Parallel sets of unheated and heated infusions were inoculated into Erlenmeyer flasks containing 70 gm. of sterile soil and 100 mgm. of nitrogen in dried blood, the moisture content being 50 per cent of the maximum. The accumulation of ammonia nitrogen after a 7-day incubation at from 20° to 22°C. is shown in table 3. A decrease in the ammonifying power of both the heated and unheated infusions obtained from subsoil layers may be noted. The average percentage of ammonia accumulated by the heated infusions for the two types of soil at the various dates and depths of sampling was 46.6 per cent for the Sassafras gravelly loam, and 39.0 per cent for the Penn shale loam.

Thus it is evident that the spores of heated infusions became active when introduced into a favorable medium. A measure of their activity when in an associative or competitive relation to the rest of the soil flora is difficult to obtain. Working with pure cultures, Conn and Bright (3, p. 329) have presented data to show that the spore-former *Bacillus cereus* is a strong ammonifier when used alone, and that the ammonia accumulation was no greater when it was mixed with the non-spore-formers, *Pseudomonas caudatus* and *Pseudomonas fluorescens*. They also found that *B. cereus* failed to appear on plates after inoculating it into a sterile manured soil with *Ps. caudatus* and *Ps. fluorescens*.

TABLE 3

The ammonia accumulation after 7 days, resulting from inoculating sterile soil containing dried blood with heated and unheated infusions of Penn shale loam and Sassafras gravelly loam

DEPTH OF SAMPLING	SASSAFRAS GRAVELLY LOAM			PENN SHALE LOAM		
	NH_3 – nitrogen accumulation		Relative accumulation by heated infusion	NH_3 – nitrogen accumulation		Relative accumulation by heated infusion
	Unheated	Heated		Unheated	Heated	
	Sampling of August 8					
cm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*
0–15	21.29	7.64	35.8	18.05	5.93	32.7
16–31	22.65	3.44	37.3	13.10	6.44	49.1
32–48	20.29	7.05	34.7	11.90	5.54	46.5
48–64	16.08	5.73	35.6	11.32	7.33	64.8
	Sampling of November 28					
0.15	8.60	4.44	51.6	11.37	4.13	36.2
	Sampling of January 9					
0.15	6.48	2.77	42.5	5.62	2.62	46.8
	Sampling of April 3					
0–15	5.89	2.61	42.6	5.65	2.62	46.9
16–31	5.40	2.29	42.4	4.07	0.69	16.9
32–48	4.10	0.77	19.0	4.38	0.58	13.2
	Sampling of June 14					
0–15	7.63	6.36	83.3	12.50	6.85	54.8
16–31	11.00	7.40	67.3	15.95	5.41	33.8
32–48	11.43	5.65	49.5	7.22	2.68	27.2
48–64	6.48	4.17	64.3	7.30	3.54	48.5
Average.....			46.6			39.0

THE RELATIVE ACTIVITY OF HEATED AND UNHEATED INFUSIONS AS SHOWN BY THE PRODUCTION OF CARBON DIOXIDE

Erlenmeyer flasks treated in the same way as those used for ammonia-accumulation tests were used for the carbon-dioxide work. Before using soil infusions a series of determinations were made on flasks inoculated with 5 cc. each of heated and unheated suspensions of *Bacillus subtilis*. These suspensions were obtained by adding sterile water to a large slant culture of the organism which had been incubated for 12 days. As may be seen in table 4, the production of carbon dioxide was as great for the last 3 days as for the second three of the 10-day period. The total carbon-dioxide production was about the same for the heated suspensions irrespective of the period of heating

TABLE 4

The ammonia-nitrogen accumulation and the CO_2 production resulting from inoculating sterile soil containing dried blood with heated and unheated infusions of Bacillus subtilis

DAYS INCUBATED	UNHEATED	INFUSIONS HEATED TO 85°C.		
		5 minutes	10 minutes	15 minutes
Carbon dioxide				
	mgm.	*mgm.*	*mgm.*	*mgm.*
0– 3	26.6	14.6	20.3	8.1
4– 7	42.0	29.9	33.1	35.6
8–10	35.3	28.0	28.3	31.5
0–10	103.9	72.5	81.7	75.2
Per cent of the production by unheated infusions		69.8	78.7	72.3
Ammonia-Nitrogen				
10	6.07	4.53	4.53	4.68
Per cent of the accumulation by unheated infusions		74.2	74.2	76.7

TABLE 5

The production of CO_2 resulting from inoculating sterile soil containing dried blood with heated and unheated infusions of Penn shale loam and Sassafras gravelly loam

DEPTH OF SAMPLING	DAYS INCUBATED	PENN SHALE LOAM			SASSAFRAS GRAVELLY LOAM		
		Unheated	Heated	Relative accumulation by heated infusions	Unheated	Heated	Relative accumulation by heated infusions
Sampling of August 8							
cm.		*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*
0–16	0–4	90.1	56.3		90.1	66.3	
	5–7	178.7	167.8		201.5	143.3	
	0–7	268.8	224.1	83.3	291.6	209.6	71.9
17–32	0.4	53.0	59.0		104.8	58.8	
	5–7	207.2	165.4		204.3	175.0	
	0–7	260.2	224.4	86.2	309.1	233.8	75.6
Sampling of June 14							
0–16	0–4	64.4	46.4		72.3	59.8	
	5–7	75.0	57.4		54.6	56.1	
	0–7	139.4	103.8	74.4	126.9	115.9	90.5
17–32	0–4	72.0	40.8		77.4	53.1	
	5–7	74.6	53.3		62.7	40.3	
	0–7	146.6	94.1	64.2	140.1	93.4	66.7
Average relative accumulation				77.0			76.2

TABLE 6

Triplicate determinations of bacterial numbers given in table 1

DEPTH OF SAMPLING	SASSAFRAS LOAM						PENN LOAM					
	Unheated inoculum			Heated inoculum			Unheated inoculum			Heated inoculum		
	1	2	3	1	2	3	1	2	3	1	2	3
Sampling of August 8												
cm.												
0– 8	1.43	1.33		0.12	0.11	0.11	11.0	12.2	9.8	0.19	0.17	0.10
9–16	3.87	2.37	3.17	0.16	0.09	0.10	4.9	7.3	4.9	0.38	0.26	0.28
17–32	3.18	3.63	3.18	0.09	0.08		1.6	1.5	2.3	0.10	0.11	0.10
33–48	1.39	1.50	1.60	0.007	0.005	0.005	0.26	0.29	0.33	0.007	0.011	0.007
49–64	1.08	0.94	0.91	0.001	0.001	0.002	0.06	0.05	0.04	0.0003	0.003	0.0002
Sampling of November 28												
0– 8	5.4	4.5	5.6	0.418	0.311	0.382						
9–16	4.9			0.441	0.370	0.323						
17–32	2.3	1.60		0.051	0.051	0.057						
33–48	0.34	0.45		0.010	0.011	0.009						
Sampling of January 9												
0– 8	6.14	5.50	7.20	0.90	0.69		29.0			1.74	2.17	2.32
9–16	4.9	4.7	4.0	1.44	1.68		24.3	27.5		3.77	5.80	
17–32	1.1	1.8	1.8	0.30	0.25	0.20	3.4	3.3	3.6	0.35	0.22	
33–48	0.20	0.20	0.12	0.002	0.002	0.001	0.32	0.30		0.007	0.007	
Sampling of April 3												
0– 8	7.7	8.2		0.32	0.33	0.39	5.8	6.1	6.9	0.25	0.23	0.32
9–16	6.2	3.5		0.17	0.11	0.13	8.0	6.5	10.0	0.29	0.33	0.32
17–32	1.4	1.6	1.4	0.038	0.033	0.030	1.4	2.2	1.7	0.90	0.10	0.10
33–48	0.81	0.64	0.75	0.002	None	None	0.45	0.27	0.27	0.003	0.003	
49–64	0.09	0.09	None	0.002	0.002	None	None	0.03	None	None	None	None
Sampling of June 14												
0– 8	3.76	3.68	4.43	0.158	0.145	0.182	12.90	16.2	17.3	0.159	0.171	0.186
9–16	2.24	2.45	2.24	0.078	0.122	0.111	7.76	6.28		0.163	0.231	0.245
17–32	0.59	0.54	0.69	0.032	0.032	0.048	5.90	6.25				
33–48				0.008	0.009	None	0.20	0.20	0.25	0.007	0.008	0.006
49–64	0.175	0.219	0.228	0.001	0.0004	None	0.26	0.25	0.23	0.0005	0.0004	0.0005

TABLE 7

Duplicate determinations of ammonia accumulations given in table 3

DEPTH OF SAMPLING	SASSAFRAS LOAM				PENN LOAM			
	Unheated infusions		Heated infusions		Unheated infusions		Heated infusions	
	1	2	1	2	1	2	1	2
	Sampling of August 8							
cm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0–15	21.88	20.70	8.02	7.25	18.8	17.30	7.40	4.46
16–32	21.72	23.50	8.30	9.17	13.85	12.35	8.15	4.72
33–48	20.98	19.60	7.73	6.36	12.46	11.33	5.86	5.22
49–64	16.40	15.76	5.60	5.86	10.05	12.60	7.77	6.88
	Sampling of November 28							
0–15	9.6	8.11	3.70	5.17	11.19	11.56	4.43	3.82
	Sampling of January 9							
0–15	6.32	6.64	3.20	2.34	5.29	6.15	2.34	2.91
	Sampling of April 3							
0–15	5.72	5.97	2.80	2.42	5.59	5.70	2.32	2.92
16–31	5.46	5.34	2.16	2.42	3.81	4.32	0.76	0.51
32–48	3.74	4.46	0.64	0.89	4.70	4.06	0.51	0.64
	Sampling of June 14							
0–15	7.95	7.30	6.61	6.10	13.30	11.70	7.50	6.20
16–31	11.50	10.39	7.50	7.30	12.90	19.00	5.54	5.28
32–48	11.75	11.11	5.40	5.90	7.50	6.94	1.21	4.14
49–64	5.91	7.05	4.00	4.34	8.70	5.90	2.50	4.64

TABLE 8

Duplicate determinations of ammonia and of carbon dioxide given in table 4

DAYS INCUBATED	UNHEATED INFUSIONS		HEATED FOR 5 MINUTES		HEATED FOR 10 MINUTES		HEATED FOR 15 MINUTES	
	1	2	1	2	1	2	1	2
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0– 3	26.6	26.6	14.7	14.4	20.3	31.2	3.2	13.0
4– 7	39.0	45.0	29.9	29.9	33.1	47.1	34.5	36.6
8–10	36.7	33.9	25.2	30.8	28.1	Lost	27.7	35.3
0–10	102.3	105.5	68.8	75.1	81.7		65.4	84.9
Ammonia-nitrogen for 10 days in flasks used for the above CO_2 determinations								
0–10	6.17	5.97	4.67	4.38	4.41	4.65	4.98	4.38

TABLE 9

Duplicate determinations of CO_2 production given in table 5

DEPTH OF SAMPLING	DAYS INCUBATED	SASSAFRAS LOAM				PENN LOAM			
		Unheated infusions		Heated infusions		Unheated infusions		Heated infusions	
		1	2	1	2	1	2	1	2
Sampling of August 8									
cm.		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0–16	0–4	92.9	87.2	69.4	63.2	72.9	107.4	60.0	52.7
	5–7	203.0	200.0	143.3	Lost	165.3	192.0	164.5	171.0
	0–7	295.9	287.2	212.7		238.2	299.4	224.5	273.7
17–32	0–4	104.8		58.8		53.0		59.0	
	5–7	204.3		175.0		207.2		165.4	
	0–7	309.1		233.0		260.2		224.9	
Sampling of June 14									
0–16	0–4	69.0	75.6	58.8	60.8	63.3	65.4	48.0	44.7
	5–7	58.2	51.0	49.4	62.7	75.3	74.6	69.0	45.7
	0–7	127.2	126.6	108.2	123.5	138.6	140.0	117.0	90.4
17–32	0–4	77.4		53.1		72.0		40.8	
	5–7	62.7		40.3		74.6		53.3	
	0–7	140.1		93.4		146.6		94.1	

between the limits of 5 and 15 minutes. This lack of effect of the period of heating was also observed in preliminary work on the numbers surviving in soil infusions. The average carbon-dioxide production by this heated suspension of *B. subtilis* was 73.6 per cent of the production by the unheated for a 10-day period.

The ammonia accumulation in the flasks from which the carbon dioxide was determined is also given in the table, and averaged 75 per cent for the heated infusions. This close correlation with the relative carbon-dioxide production has been previously shown for bacterial cultures (9, p. 242).

Table 5 gives the production of carbon dioxide from infusions of the two types of soil for the sampling of June 14 and August 8. The average per cent of carbon dioxide by the heated infusions of Penn shale loam for a 7-day period was 77 per cent of the production by the unheated infusions. The corresponding value for the Sassafras gravelly loam was 76.2 per cent.

It is believed that the higher production for the samples of August 8 was due to a higher temperature of incubation, as the refrigeration apparatus was not working properly during the month of August, and the incubation temperature was higher than from 20° to 22°C.

CONCLUSIONS

1. Infusions from the five successive layers of the upper 64 cm. of soil were heated to 85°C. for 10 minutes, and the carbon-dioxide production, ammonia accumulation and numbers were compared with a similar treatment with unheated infusions.

2. In the surface soil of the finer-textured loam, 5.8 per cent, and of the coarser loam, 8.7 per cent, of the original count survived a temperature of 85°C. for 10 minutes.

3. The relative number surviving in the subsoil was much less, being 2.7 and 1.6 per cent, respectively.

4. Very few fungus colonies, and no actinomycetes or chromogenic types were observed in the plates inoculated with heated infusions.

5. For a 7-day period the average ammonia accumulation resulting from inoculating with heated infusions was 46.6 per cent of that with unheated infusions obtained from Sassafras gravelly loam and 39 per cent when infusions from Penn shale loam were used.

6. For a 10-day period the carbon dioxide produced by inoculating with heated infusions of *B. subtilis* was about the same whether the infusion had been heated for 5, 10, or 15 minutes, and averaged 73.6 per cent of the amount produced by unheated infusions.

7. For a 7-day period the average carbon-dioxide production resulting from the use of heated infusions of Penn shale loam was 77 per cent of that obtained with unheated infusions. The corresponding value for Sassafras gravelly loam was 76.2 per cent.

8. The accumulation of NH_3 and the production of carbon dioxide resulting from inoculating a sterile soil with heated infusions correlate with each other in showing that the bacterial spores of the soil are capable of energetic activity when supplied with sufficient food and moisture. As to whether they would be equally active in liberating plant-food when competing with the actinomycetes, non-sporulating bacteria, and fungi in a normal soil flora, is a subject which needs further investigation. When sufficient information concerning the importance and environmental requirements of the various groups of soil microörganisms has been obtained, the way will be ready for a more rational control of soil conditions to enhance the action of the beneficial groups and to check those which are detrimental.

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STUDIES ON THE REACTION OF PLANT JUICES[1]

A. R. C. HAAS[2]

Departments of Agricultural Bacteriology and Soils, University of Wisconsin

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Comparatively little investigation has been done on the actual reaction[3] of plant juices upon plant metabolism, notwithstanding its importance. Loeb, in 1919, in a series of five papers on amphoteric colloids in the Journal of General Physiology, has shown the importance of the reaction upon the retention and excretion of ions from protein-containing solutions, as well as upon other physical and chemical properties of amphoteric colloids. The factors that may affect the reaction of plant juices are numerous, and as yet are but little understood.

Inasmuch as the soil reaction is one of the important factors affecting the growth of plants, it is quite possible that the degree of acidity of plant juices may be correlated with the reaction of the soil upon which the plants are grown. It has already been shown by Fred and Davenport (4) that the reaction of the medium markedly affects the metabolism of legume bacteria, and that they are rendered inactive at a more or less well-defined critical pH value of the medium. In addition to the reaction of the substrate, meterological and hereditary factors may be considered as possibly affecting the reaction of plant juices.

The present paper deals with the actual and the total acidities and the total alkalinity of the juices of a number of plants of agricultural importance, together with a study of the influence of liming the soil upon these acidities. The actual acidities of the different parts of plants and their relation to one another are discussed. The effects of age, lack of chlorophyll and changes in illumination, upon the actual and total acidities of plant juices also are considered.

The actual acidity of soil solutions (27) from which plants derive food has been the subject of considerable investigation (25). The studies of Hoagland (12) on the relation of the nutrient solution to the composition and reaction of the cell sap of barley plants, is a step in the right direction.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] The writer has been obliged to conclude his present studies and finds it advisable to present the data thus far obtained.

[3] By actual acidity is meant the amount of dissociated acid. The total acidity includes both the dissociated and undissociated acids.

It has been shown that the actual reaction of the juice of most plants is markedly acid (7, 8), and that only occasionally are species found with cells the juices of which are neutral or slightly alkaline in reaction (5). However, Kappen (13) has recently reported a slight alkalinity in the juice of wheat roots. Rose (20) has also reported the interesting observation that as germination begins, the reaction of the embryos of *Sambucus* changes from alkaline to acid but that the endosperm remains alkaline. The juice of succulent plants has been found by Hempel (11) to be quite acid in reaction, and it was she who first elucidated the nature of the buffer processes existing in the juice of such plants.

For previous work upon reaction as regards both plants and animals, the bibliography compiled by Schmidt and Hoagland (21) is very complete.

METHOD OF DETERMINING THE ACIDITY OF PLANT JUICES

The determination of both the actual and the total acidity (10) of plant juices was carried on by the use of the hydrogen-electrode. It was found that the juice obtained from the plants growing in a single pot was frequently insufficient for carrying on a determination of the actual reaction when a 3-by-10-cm. glass titration tube was used. An effort was made to use the indicator paper method (9) but it was found quite impossible to secure clear or nearly clear plant juice even after filtration by various methods and repeated centrifuging. Unless the juice was very acid, it retained a deep green color. Moreover, it was considered inadvisable to use two methods (the indicator paper method is less accurate than the electrometric) in making comparative determinations, especially in case differences are small.

Accordingly a small hydrogen-electrode vessel was devised as shown in figure 1, in order that determinations of the actual acidity could be made when only three or four drops of plant juice were available. The essential point in the construction of the apparatus is to make the bulb F of such size and shape that a few drops of solution suffice to cover the lower end of the glass tube for the KCl contact and gas-electrode. The small hydrogen-electrode vessel was found to be more convenient than the larger vessel except when the juice was to be titrated, in which case the larger vessel was used. A small quantity of plant juice was found to be preferable to a larger quantity, for with small amounts of juice equilibrium was more rapidly attained. The results obtained with the small apparatus were compared with the results obtained with the larger hydrogen-electrode vessel.

The juice from fresh medium red clover grown out-of-doors was obtained by first crushing the cells in a tinned meat grinder and then pressing out the juice through a clean linen cloth. The extracted juice was not centrifuged. Four drops of the plant juice (0.12 cc.) were introduced with a pipette into the small hydrogen-electrode vessel. The actual reaction of the plant juice was found to be pH = 6.02. The original juice was left standing ½ hour

and a second quantity amounting to 4 drops (0.13 cc.) was used. The actual reaction was found to be pH = 6.06. A 7-cc. sample of the original juice was then run with the larger hydrogen-electrode vessel and the actual reaction of the juice was found to be pH = 6.06. In the determination of the actual

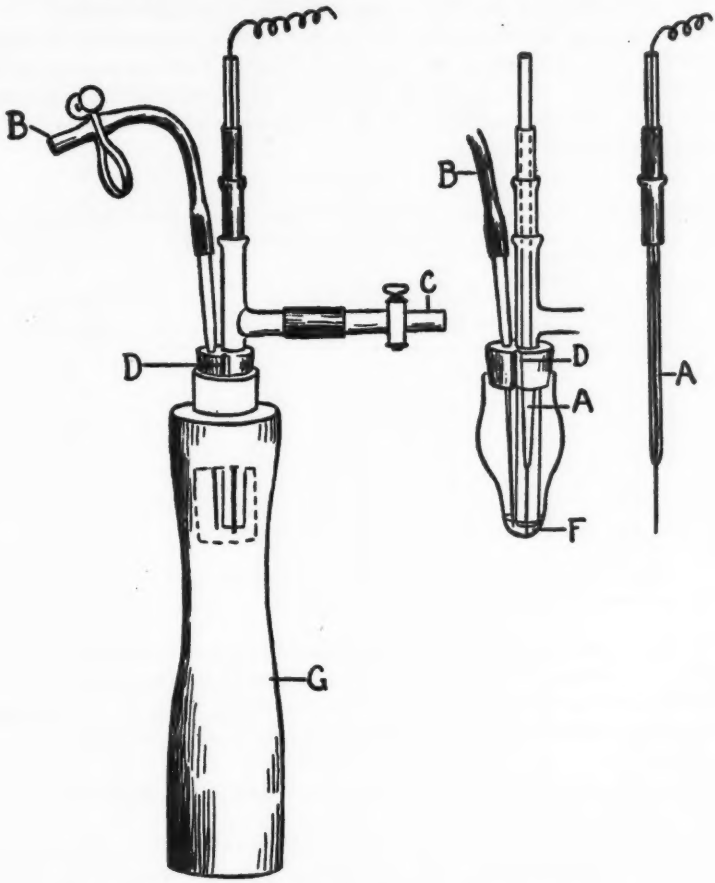

FIG. 1. THE SMALL HYDROGEN ELECTRODE APPARATUS DRAWN TO SCALE

A, Hydrogen-electrode wire at lower end connected with mercury to wire connection at upper end. *B*, Connection with KCl to the calomel electrode. *C*, Hydrogen inlet. *D*, Holes for outlet excess gas. *F*, Plant juice. *G*, Rubber holder for hydrogen electrode vessel with glass tube inserted at lower end for rigidity, and window at upper end for observation.

reaction of some very minute quantities of plant juice, this small type of hydrogen-electrode apparatus served a very useful purpose and facilitated considerably the numerous determinations of the reaction made upon agricultural plants.

Besides determining the actual reaction of plant juice, it was found possible, whenever 5 cc. or more of juice was available, to titrate the total acid by means of the hydrogen-electrode. It is possible to titrate much smaller quantities of juice, but it is not convenient. By titrating the total acidity of the plant juice it is possible to obtain an idea of its buffer action.

The following titrations, taken at random from a large number of determinations, serve to indicate the degree of accuracy that can be secured by the electrometric titration (10) of aliquots of the same sample of expressed plant juice when the readings of E. M. F. are made with a voltmeter. The results are given in table 1.

TABLE 1

Agreement of duplicate determinations of actual acidity upon adding 0.0689 N sodium hydroxide to 10 cc. of juice of medium red clover tops

FIRST TRIAL		SECOND TRIAL	
Actual acidity	NaOH added	Actual acidity	NaOH added
pH	*cc.*	pH	*cc.*
6.08	0	6.10	0
6.92	1.0	6.88	1.0
7.53	2.0	7.53	2.0
8.09	3.0	8.07	3.0
8.41	4.0	8.41	4.0
8.69	5.0	8.68	5.0
9.00	6.0	9.02	6.0
9.34	7.0	9.34	7.0
9.63	8.0	9.60	8.0

Without plotting the values, it is obvious that the two successive titrations check very closely. In an electrometric titration, it is frequently possible to observe errors in the reading of the burette or the E. M. F. by the fact that such points ordinarily do not fall close to the trend of the titration curve.

EFFECT OF STANDING ON THE ACTUAL AND TOTAL ACIDITIES

The previous treatment of the plants and the time elapsing between expressing and using the plant juice may influence the actual and total acidities obtained. The total acidity of plant juice will differ according to the pH we select as our end-point. If we select pH = 6.81 we have the turning point for litmus; if we select pH = 8.31 we have the turning point for phenolphthalein. Hence in titrating for total acidity we can tell at once from the curve the amount of alkali required for titration to any given pH and consequently to the color change for any indicator. For example, some medium red clover plants 12 to 14 inches high, growing on a limed plot (pH = 7.53) were cut off just above the surface of the soil at 11.30 a.m. and

TABLE 2

Effect of standing on actual and total acidities of medium red clover juice
10 cc. of juice titrated with 0.0684 *N* sodium hydroxide

ACTUAL ACIDITY	PLANTS FROM LIMED SOIL	ACTUAL ACIDITY	PLANTS FROM UNLIMED SOIL
I (1.50 p.m.)		I (8.15 p.m.)	
pH	*cc.* NaOH *added*	pH	*cc.* NaOH *added*
6.12	0.0	5.94	0.0
7.31	1.0	6.45	1.0
8.05	2.0	6.94	2.0
8.56	3.0	7.53	3.0
8.90	4.0	8.07	4.0
9.20	5.0	8.39	5.0
9.46	6.0	8.75	6.0
II (3.05 p.m.)		II (9.30 p.m.)	
6.12	0.0	5.82	0.0
6.84	1.0	6.45	1.0
7.87	2.0	6.94	2.0
8.22	3.0	7.33	3.0
8.41	4.0	7.97	4.0
8.98	5.0	8.22	5.0
9.35	6.0	8.66	6.0
III (3.55 p.m.)			
6.03	0.0		
6.84	1.0		
7.56	2.0		
8.00	3.0		
8.36	4.0		
8.68	5.0		
9.05	6.0		
IV (5.00 p.m.)			
6.04	0.0		
6.72	1.0		
7.51	2.0		
7.88	3.0		
8.34	4.0		
8.69	5.0		
9.08	6.0		
V (7.10 p.m.) Freshly expressed and centrifuged			
6.10	0.0		
6.72	1.0		
7.39	2.0		
7.97	3.0		
8.69	5.0		
9.02	6.0		

placed in beakers containing water. Some medium red clover plants 8 to 10 inches high from an unlimed but otherwise similarly fertilized plot (pH = 6.19) were treated in the same way. Just prior to the first determination in each of the two series of table 2, the lower portion of the plants that had been in the beakers of water were cut off and discarded, and the juice of the upper remaining portion was expressed and centrifuged 15 minutes. The juice of the plants from the limed soil was kept in the dark and 10-cc. aliquots were titrated at intervals throughout the afternoon. The juice of the plants from the unlimed soil was not expressed until in the evening and only two successive titrations were made.

From the table it is evident that on standing in the dark there is a tendency for the medium red clover juice to undergo changes in its actual and total acidities. In the first four determinations on equal aliquots of the same original juice of plants grown on the limed soil there was an increasing total acidity, whereas the fifth determination, made upon freshly expressed and centrifuged juice, behaved more like determinations III and IV than like I and II as regards total acidity and more like determinations I and II than like III and IV as regards initial actual acidity. The juice of the unlimed clover had a greater actual and total acidity, upon allowing the expressed and centrifuged juice to stand in the dark, than when used immediately. When the juice of the limed clover plants was left standing over night for 24 hours and its actual acidity again determined, it was found that the value for the actual acidity had changed from pH = 6.12 to pH = 5.91. This marked increase in actual reaction upon oxidation of the juice is in accord with the observations of Miss Hempel (11) for the juice of succulent plants. The results in table 2 cannot be compared with those in table 1 as it is essential to use fresh plants. Furthermore, as will be indicated, the soil solution and other factors may affect the actual and total acidities of plants.

EFFECT OF LIMING ON THE ACTUAL AND TOTAL ACIDITIES

On comparing determination I of the unlimed clover, in table 2, with determinations I and V of the limed clover, it is found that the unlimed clover has a greater actual and a greater total acidity than the limed clover, regardless of the pH which might be chosen as the end-point.[4]

[4] In order to compare determination I of the unlimed with determination V of the limed clover as regards total acid, we select any pH as our turning point and note the amount of alkali required to bring the juice to the selected pH end-point. Thus for example, in determination I of the unlimed clover we find that 5 cc. of alkali are required in order to bring the pH of the juice to 8.39, whereas in determination V of the limed clover less than 5 cc. of the alkali is required in order to bring the pH of the juice to 8.39, since 5 cc. carries us 0.30 beyond the end-point pH = 8.39. This shows that the juice of the unlimed clover has a greater total acidity than the juice of the limed clover with reference to pH = 8.39 as our end-point.

It seems important, therefore, to consider the effect of liming the soil on the actual and total acidities of plants grown on such soils. Various crops were grown on Plainfield sand, using pots containing 11,500 gm. of soil. Plant tissue was available from these pots through cooperation with Dr. C. B. Clevenger.

Half of the pots received sufficient ground limestone to neutralize all of the acid as indicated by the Truog test (24), and all the pots received 2 gm. of dipotassium phosphate. The seeds were planted March 1.

The actual and the total acidities of 6 cc. of alsike clover juice were determined for both the limed and the unlimed Plainfield sand. The tops were cut May 13 and the juice was centrifuged for 10 minutes after being expressed. In none of the experiments in this paper was there any water added to the crushed plant tissue or the expressed juice. The values obtained for alsike clover are given in the form of curves in figure 2.

From the curves, it is evident that the actual as well as the total acidities of the juice of the tops of alsike clover plants grown without liming are greater in every case than those with liming. If, for example pH = 8.0 is chosen as the end-point, it is seen at once that the 6-cc. sample of juice from the plants with liming required 2 cc. of alkali, whereas that from plants without liming required 3 cc. to bring the juice to pH = 8.0.

The 7-cc. samples of juice of serradella tops cut on June 2 from limed and unlimed Plainfield sand gave a different relationship (figure 2) from that described for alsike clover. The actual acidity of the plants with liming was greater than that of the plants without liming. As alkali was added, at first the total acidity of the juice of the limed plants is greater, then less, and then greater than that of the juice of the unlimed plants.

When the 8-cc. samples of juice of the tops of garden bean plants, grown on limed and on unlimed Plainfield sand and cut on May 13, were examined, the results represented by the curves in figure 2 were obtained. It is found that the actual as well as the total acidities of the juice of plants with liming was greater than that of the plants without liming. These results might be explained possibly by such a fact as Gile and Ageton (6) have found for beans, namely, that in the ash of bush beans the amount of lime was slightly decreased rather than increased with increasing amounts of calcium carbonate in the soil.

Barley plants, not yet in bloom, were cut off at the surface of the limed and unlimed Plainfield sand on May 12. The expressed plant juices were centrifuged for 15 minutes. The results obtained for the actual and the total acidities of 15-cc. samples of the juices are plotted in figure 3. The initial actual acidity was greater in the plants from unlimed than from limed soil. The total acidity in both cases was practically the same for any given pH as the end-point.

When 15-cc. samples of juice obtained from oat plants cut May 9 on limed and unlimed Plainfield sand were examined, it was found (fig. 3) that

the initial actual acidities of the juices were practically the same. However, as regards total acidity, it was found that no matter which pH was chosen as the end-point, the plants with liming showed a greater total acidity than those without liming.

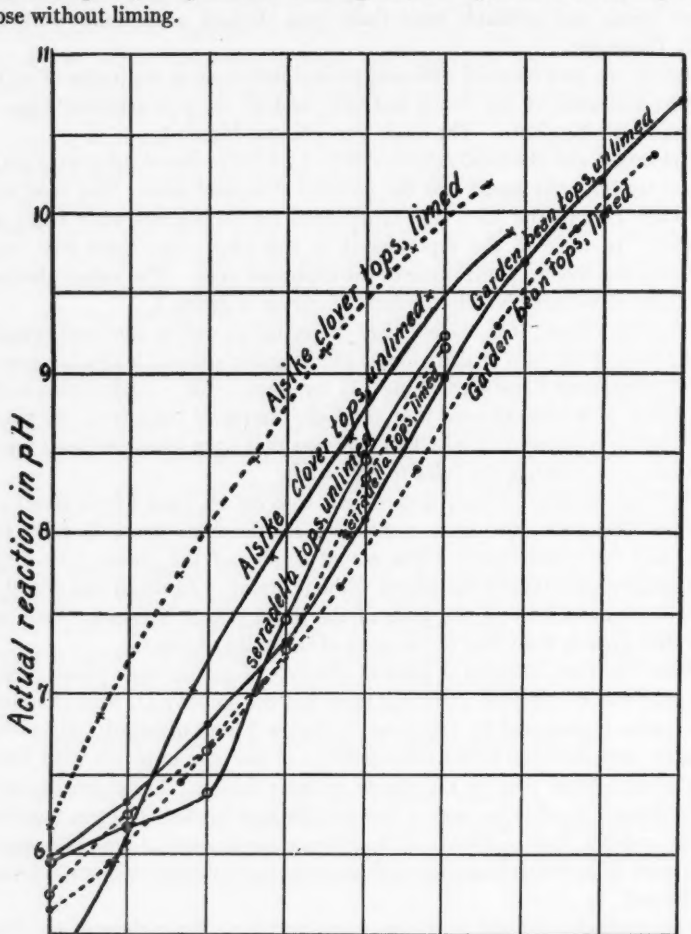
Actual reaction in pH
Cubic centimeters of .0684N. Na OH added
Alsike clover tops, limed
Alsike clover tops, unlimed
Garden bean tops, unlimed
Garden bean tops, limed
Serradella tops, unlimed
Serradella tops, limed

FIG. 2. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES (EXPRESSED IN TERMS OF CUBIC CENTIMETERS OF 0.0684 *N* SODIUM HYDROXIDE) OF THE JUICES OF PLANTS GROWN ON LIMED AND UNLIMED SOIL

Corn plants growing on soils similar to those of the oat plants were cut May 9. The titration curves of 15-cc. samples of both juices resemble those for oat plants. The initial actual acidities with and without liming were the same. However, for any chosen end-point (pH) the total acidity of the juice of the corn with liming was greater than that without liming.

Truog (25) has suggested that one of the chief functions of calcium, when taken up by the plant in the carbonate or bicarbonate form, is to neutralize and precipitate acids within the plant and thus prevent an injurious degree of acidity. How these irregular results may be harmonized with this suggestion will now be discussed.

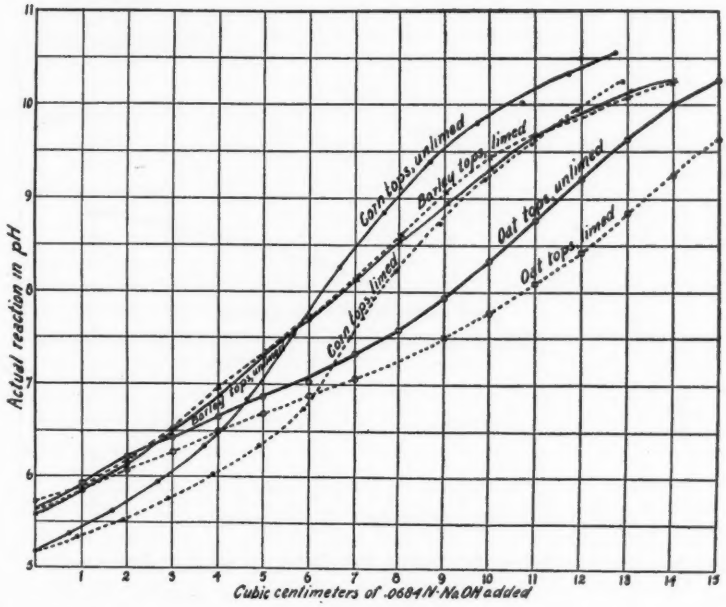

FIG. 3. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF BARLEY, OAT, AND CORN PLANTS GROWN ON LIMED AND UNLIMED SOIL

Some of the results from plants grown upon Plainfield sand are most irregular if not abnormal when compared with those from plants grown upon other types of soil. Frequently the addition of lime to Plainfield sand had an unfavorable effect on plant growth even with plants that ordinarily are benefited by liming. This soil thus gave some unexpected results.

In the experiments just described there was not taken into consideration the possibility that differences in actual and in total acidities may exist in different parts of the same plant. With succulent plants in which the leaves are of large size it is relatively a simple matter to separate the leaf, stem, and

root portions of the plants and to compare the actual and the total acidities of corresponding parts. With alfalfa, clover and other plants of a similar nature, it requires considerable time and patience to separate the leaves, stems, and roots from one another in order to obtain sufficient of the plant juice with which to carry on the determinations.

In some of the determinations of the actual acidity of the plant juice of rhubarb leaf-stalk, it was found (8) that the actual reaction varied considerably according to the part of the plant from which the juice was expressed. The observations of Rose (20) upon the reaction of different parts of seed tend to emphasize the fact that it is essential to compare the expressed juices of corresponding parts of plants.

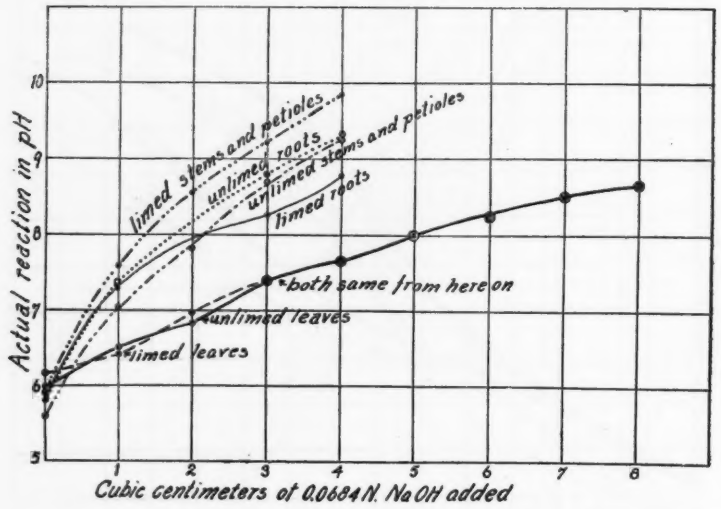

FIG. 4. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF LEAVES, STEMS, PETIOLES, AND ROOTS OF MEDIUM RED CLOVER PLANTS GROWN WITH AND WITHOUT LIMING

The results reported in this paper (fig. 4) point out the fact that leaves, stems, and roots may differ quite considerably in their actual reaction.

In order to control the conditions as much as possible, fresh medium red clover was secured from the adjoining farm plots; one plot had received lime, the other had not. The actual reaction of the limed plot (aqueous extract) was pH = 7.53 and that of the unlimed plot pH = 6.19. The plants from the limed plot were 20 to 24 inches high, whereas those from the unlimed plot were 10 to 18 inches high. When the plants were cut on May 30 those from the unlimed plot appeared a lighter green than the others. In the first determination the leaflets were picked from each plant and their juice expressed

in the usual manner. In order to observe errors that might be introduced as a result of changes in the juice during a 20-minute period of centrifuging, a determination of the actual acidity of a portion of the juice was made immediately after the juice was expressed, whereas the remainder of the juice was centrifuged. The results obtained for the effect of centrifuging on the initial actual acidities of the juices may be stated briefly as follows:

Uncentrifuged

Limed plants
- Leaves..... pH = 6.19
- Stems and petioles..... pH = 5.95

Unlimed plants
- Leaves..... pH = 6.02
- Stems and petioles..... pH = 5.63

Centrifuged

Limed plants
- Leaves..... pH = 6.12
- Stems and petioles..... pH = 5.89

Unlimed plants
- Leaves..... pH = 5.99
- Stems and petioles..... pH = 5.62

Obviously, only a slight increase in actual reaction of the juice of the aboveground parts occurred during the centrifuging.

The curves (fig. 4) represent the actual and the total acidities (expressed in terms of 0.0684 *N* sodium hydroxide) of the 7-cc. samples of different parts of medium red clover plants. The titration curves make it evident that the initial actual reaction of the leaves, stems and petioles, and roots of plants from limed soil was less than that of the corresponding parts of the plants from unlimed soil. The total acidities of the leaf juices were quite similar in both cases. The juice of the stems and petioles of the plants from limed soil had a smaller total acidity than that of the plants from unlimed soil. The total acidity of the roots without liming was at first greater than that with liming, but became smaller than that with liming as the titration proceeded.

However, the day previous to the above experiment, two lots of medium red clover roots were secured from the same plot as before and determinations made of the actual reactions of the uncentrifuged juice. From table 3 it is evident that the roots with liming had a slightly greater actual acidity than the roots without liming and that the results obtained for the root juice (fig. 4) are reversed.

Timothy and winter wheat plants were cut June 11 at the surface of the soil of the same limed and unlimed plots in which the medium red clover was growing. The actual reactions recorded in table 3 indicate that the effect of liming the soil was to decrease the actual acidity of the juice of timothy and wheat.

White mustard plants were grown on the limed and the unlimed Plainfield sand from March 1 to June 13. When the tops were cut off they were very unequal in size on the limed and unlimed pots. The roots from the limed and unlimed soil with about an inch of the attached stem were the only parts utilized in the determinations of the actual acidities of the juices.

Table 3 makes it clear that the juice of the roots with liming showed a less actual acidity than without liming. Whenever roots were used they were repeatedly washed with pure distilled water and then shaken free of any adhering moisture, after which they were further dried by drawing them

TABLE 3

Actual acidities of juice of different parts of plants grown on limed and unlimed soil

PLANT	PART OF PLANT	TREATMENT	LIMED SOIL	UNLIMED SOIL
			pH	pH
Medium red clover, lot 1	Roots	Uncentrifuged	5.87	5.91
Medium red clover, lot 2	Roots	Uncentrifuged	5.82	5.88
Timothy	Leaves, stems, young spikes	Uncentrifuged	6.17	6.14
Timothy	Leaves, stems, young spikes	Uncentrifuged	6.19	6.12
Winter wheat, lot 1	Tops (pollen stage)	Centrifuged 10 minutes	6.33	5.95
Winter wheat, lot 2	Leaves, stems, no spikes	Centrifuged 10 minutes	6.12	5.77
White mustard	Roots with lower 1 inch of stem	Uncentrifuged	5.91	5.62
White mustard	Entire plants	Uncentrifuged	5.78	5.48
Corn	Tops	Centrifuged	5.48	5.48
Field peas	Tops	Uncentrifuged	6.53	6.80
Buckwheat	Entire plants	Uncentrifuged	5.97	5.48
Alfalfa	Tops	Centrifuged	6.19	5.99
Baltic alfalfa no. 550	Roots	Uncentrifuged	6.12	6.21
Common South Dakota alfalfa, no. 363	Roots	Uncentrifuged	6.12	6.06
Alsike clover	Roots	Uncentrifuged	5.84	5.68

over a good grade of filter paper. These determinations were made possible by using the small hydrogen-electrode vessel. The tops were so unequal in size (plate 1) that it seemed best to utilize the tops of white mustard at an earlier stage, when they were more nearly equal in size and a more fair comparison of the actual acidities of the juices would be possible.

White mustard seed was sown in Colby silt loam in pots containing 11,500 gm. of the soil. To some pots no lime was added; to others 1 per cent sufficient ground limestone was added, to neutralize the acidity as shown by Truog's method. Each pot received 25 cc. of water containing 1 gm. of dipotassium phosphate. The other plants grown on Colby silt loam, which are to be mentioned later, received the same soil conditions as white mustard.

The juice of the white mustard plants was so small in amount that in order to obtain any at all, it was necessary to place the entire plants inside a linen cloth and after crushing the plant cells in a porcelain mortar, the juice was expressed by wringing the cloth. The actual acidities of the juices of the plants under both treatments, as regards liming, are given in table 3. Even at this early stage in the growth of the plants, a distinct decrease in the actual reaction of the juice of plants with liming as compared with that of plants without liming is evident.

When corn plants about 10 to 12 inches high that were grown on limed and on unlimed Colby silt loam were used for determinations of actual acidities,

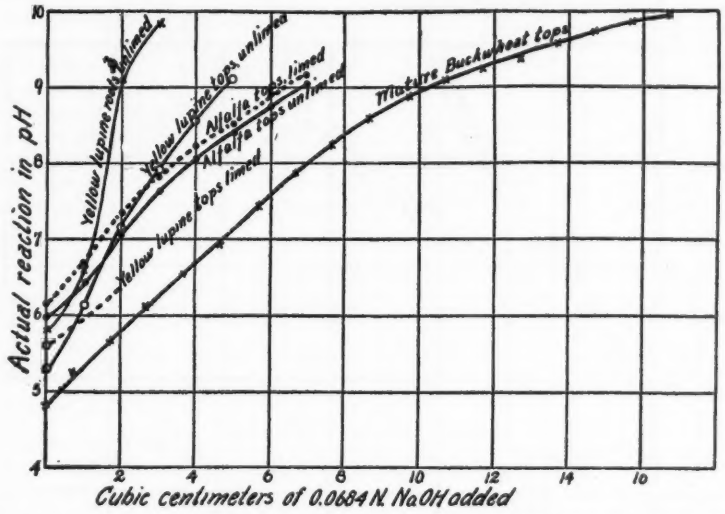
Actual reaction in pH
Cubic centimeters of 0.0684 N. NaOH added
Yellow lupine roots limed
Yellow lupine tops unlimed
Alfalfa tops limed
Alfalfa tops unlimed
Yellow lupine tops limed
Mature Buckwheat tops

FIG. 5. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF BUCKWHEAT TOPS AND OF THE JUICES OF ALFALFA TOPS, LUPINE TOPS, AND LUPINE ROOTS GROWN WITH AND WITHOUT LIMING

the juice in both cases gave a value of pH = 5.48. The small hydrogen-electrode vessel was not available at the time and separate determinations on the leaves and stalks were not made.

The actual acidities of the juices of field pea tops and of entire buckwheat plants grown on limed and unlimed Colby silt loam were determined. The field pea plants in each case were about 8 inches high; the buckwheat plants in each case were about 5 to 6 inches high. The results are given in table 3.

In table 3 it is seen that the acidity of the juice of the pea plants with liming was greater than that of the plants without liming. With buckwheat the converse was true, and the actual reaction with liming was much less than without liming.

Plants from limed and unlimed plots at Monroe, Wisconsin, were brought into the laboratory shortly after being dug up. The roots were well covered with soil and the tops were in excellent condition. Table 3 gives the results for alfalfa tops and several lots of roots, obtained from limed and unlimed plots. Figure 5 gives the titration curves of 9.5-cc. samples of juice of the alfalfa tops from limed and unlimed soil and shows that liming decreased both the actual and total acidities.

Viewing table 3 as a whole, it is found that in one case, liming the soil was followed by no change in the actual acidity of the plant juice. However, in 10 out of 14 cases the addition of lime was followed by a decrease in the actual acidities of the plant juices.

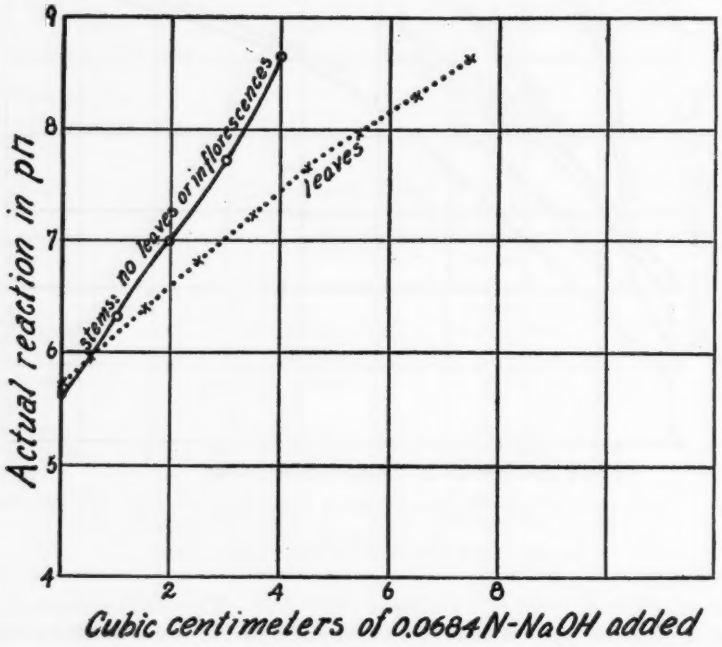

FIG. 6. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF THE JUICE OF THE LEAVES AND STEMS OF ORCHARD GRASS

Although yellow lupines do not grow well on limed Plainfield sand, it was possible to secure sufficient of the plant juice to obtain an idea concerning the effect of liming on the actual acidity of the juice. The plants had grown from March 1 to June 3. The plants without liming were in bloom, whereas the plant with liming was not in bloom and was only about one-half as high as the former. Unfortunately, insufficient juice was obtained from the single plant grown on limed soil to carry on a titration for the total acidity.

In both cases the buds, flowers, and nodules were discarded. A 7-cc. sample of juice was used in each titration. The results obtained for yellow lupine are given in the form of curves in figure 6.

The curves indicate that lime decreased the actual acidity of the juice of yellow lupine tops. Without liming the total acidity of the juice of the tops was greater than that of the roots.

TABLE 4

A summary of the effect of liming the soil upon the actual and total acidities of plant juices

PLANT	ACTUAL ACIDITY	ACTUAL ACIDITY OF JUICE		TOTAL ACIDITY
		Limed	Unlimed	
		pH	pH	
Alfalfa tops	Decrease	6.19	5.99	Decrease
Alfalfa roots (Baltic no. 550)	Increase	6.12	6.21	
Alfalfa roots (Common South Dakota no. 363)	Decrease	6.12	6.06	
Alsike clover roots	Decrease	5.84	5.68	
Alsike clover tops	Decrease	6.19	5.28	Decrease
Barley tops	Decrease	5.72	5.62	No difference
Buckwheat (entire seedlings)	Decrease	5.97	5.48	
Corn tops	No difference	5.19	5.19	Slight increase
Corn tops	No difference	5.48	5.48	
Field peas tops	Increase	6.53	6.80	
Garden bean tops	Increase	5.65	5.97	Increase
Lupine, yellow tops	Decrease	5.63	5.31	
Lupine, yellow roots			5.80	
Medium red clover roots	Increase	5.87	5.91	
		5.82	5.88	
Medium red clover tops	Decrease	6.12	5.94	Decrease
Medium red clover leaves	Decrease	6.19	6.02	No difference
Medium red clover stems and petioles	Decrease	5.95	5.63	Decrease
Medium red clover roots	Decrease	5.92	5.84	Decrease then increase
Mustard, white, roots	Decrease	5.91	5.62	
Mustard, white entire plants	Decrease	5.78	5.48	
Oat plants	Decrease	5.67	5.65	Slight increase
Serradella tops	Increase	5.74	5.94	Decrease then increase
Timothy tops	Decrease	6.17	6.14	
		6.19	6.12	
Winter wheat tops	Decrease	6.33	5.95	
Winter wheat, leaves and stems, no heads	Decrease	6.12	5.77	

Table 4 gives the results of liming the soil upon the reaction of plant juices. Apparently most of the evidence at hand confirms Truog's suggestion that lime may function to decrease or regulate the actual acidity of the plant protoplasm.

The experiments of Kappen (13) emphasize the fact that as the actual acidity increases in the root juice, there is also a very appreciable increase in the total acidity of the juice. In the titration of the total acidities for limed and unlimed conditions, the writer found that when liming caused a decrease in the actual acidity, frequently there was a decrease in the total acidity; also that when liming produced an increased actual acidity, there was usually an increased total acidity. In this paper, the data for the effect of liming on the total acidities of plant juices are too meager to permit making a conclusive statement regarding it. That the total acidities of plant juices do sometimes fluctuate quite regularly in the same direction as the actual acidities, will be shown in a subsequent paper by Bauer and myself. Miss Irwin (16) has made the interesting observation that under certain conditions the carbon-dioxide production by petals may increase and still the actual reaction of the cell contents may decrease.

AGRICULTURAL PLANTS WITH HIGH ACTUAL AND TOTAL ACIDITIES

As stated the actual reaction of the juice of yellow lupines was found to be pH = 5.31 and pH = 5.63 for unlimed and limed conditions, respectively. Hempel (11) working with 20-day-old white lupine seedlings, found their actual reaction to be pH = 5.93. Of the plants with quite acid juices, buckwheat is of interest. It was mentioned before that the juices of buckwheat (entire seedlings 5 to 6 inches high with but few leaves on each seedling) had actual reactions of pH = 5.48 and pH = 5.97 when the plants were grown on unlimed and limed soil, respectively. Buckwheat tops, partially flowering and in seed, gave a juice with an actual acidity of pH = 4.82. An aqueous extract of the soil on which this buckwheat was grown had an actual reaction of pH = 7.68. The actual acidity of the juice of nearly mature buckwheat plants is markedly greater than during the early stages of growth. The titration values for the actual and the total acidities of the juice of buckwheat plants in their advanced stage are plotted in figure 5.

The curve for buckwheat indicates a marked buffer action of its juice. The 15-cc. sample of the centrifuged juice required the addition of about 4.2 cc. of the alkali to bring the juice to pH = 6.81, which is the color change for litmus, and about 8 cc. of the alkali to bring the juice to pH = 8.31, the color change for phenolphthalein.

VARIATIONS OF REACTION IN DIFFERENT PARTS OF PLANTS

The titration curves, in figure 4, for medium red clover, from limed and unlimed soils, show that the actual acidities and the acid reserves of the juices of the leaves, stems, and roots may differ quite considerably. By acid reserve we mean the amount of dissolved acid or acid salts in the undissociated form that dissociates as we neutralize some of the acid (hydrogen ions). The curves in figure 4 show that for both soils the leaf juice had the

greatest acid reserve. The acid reserve of the root juice, however, was closer to that of the stems and petioles than to that of the leaves, and was somewhat intermediate in position. The results of Kappen (13) indicate that the juice of the above-ground parts of a plant has a greater total acidity than that of the roots.

The importance of determining the actual and total acidities of comparable tissues or organs, or parts of the plants, rather than of the entire tops, is further emphasized in the experiments with sweet clover. It is evident that if plant juice varies in its actual or total reaction according to the part of the plant from which it is expressed, then the number of leaves, stems, roots, etc., on the two lots of plants that are being compared may become a factor of considerable importance.

In order to study the variations of actual reaction in different parts of plants, the results obtained for blue-grass may be examined. The plants were about 20 inches high and in the pollinating stage. The soil reaction was neutral to slightly alkaline. The actual acidities of the uncentrifuged juices of the upper five inches of the tops which included all of the inflorescences, and of the lower 15 inches of the tops, were determined and found to be as follows: upper 5 inches of tops including inflorescences, pH = 6.11; lower 15 inches of tops, pH = 5.92. The results for blue-grass show that plant juice may vary in its actual reaction in different parts of the plant.

The results with orchard grass also show a change of actual reaction of the juice in different parts of the plant. The orchard grass was cut June 9 from a neutral to slightly alkaline soil. Determinations were made of the actual reaction of the different parts of the plants, using 10-cc. samples of juice centrifuged for 10 minutes. The curves in figure 6 plotted from the results obtained, show that the initial actual acidity of the juice of the stems is greater than that of the leaves. Between pH = 6 and 9 the total acidity of the leaf juice is greater than that of the stem juice. Until approximately the point pH = 6 is reached, the conditions of total acidities are reversed from what they are beyond pH = 6.

IS THERE A GRADIENT OF REACTION IN PLANTS?

An extreme case, that of sweet clover, may be taken to emphasize the differences that exist in the reaction of the juice from different parts of the same plant. At 3:45 p.m., May 29, an entire sweet clover plant was dug up. The actual reaction of the soil extract was pH = 7.68. The tops were 24 inches high and the length of thickened root obtained was about 8 to 10 inches. The tops represented the second year's growth. The sunlight was intensely bright and the leaves had lost some of their turgidity. The actual acidity determinations of the juice taken from different portions of the same plant are given in table 5. These results indicate that the juice in this particular plant was acid, neutral, and alkaline, depending upon the portion of

the plant from which the juice was taken. Among the numerous plants examined, the writer has not found another species exhibiting this condition. It is evident from table 5 that in this case a steep gradient existed in the actual reaction from the one end to the other, the upper end being the least acid. The studies of Child (2) on the metabolic and susceptibility gradients in plants and animals are of great interest, and possibly the use of sweet clover in these studies would give further valuable information. At present our results upon acidity gradients are too meager to permit of conclusions regarding their significance.

In connection with acidity gradients in plants it is well also to consider their relation to enzyme activity (3, 17), since the reaction within a plant may range from marked acidity at one end to a considerable alkalinity at the other end. Bunzell (1) has reported the results of a thorough study of the degree of oxidase activity of different plant juices, but has found it necessary to carry on all of the experiments in a solution, the actual acidity of which was greater than that of the neutral point for distilled water. In alkaline solutions, pyrocatechol, as well as most other good oxidase reagents, are oxidized by atmospheric oxygen, so that it is quite impossible to distinguish between the oxidation due to the alkalinity of the solution and that due to the oxidase activity of the plant juice.

In order to demonstrate conclusively that a large plant like this can possess an alkaline reaction of the degree found at one end of the acidity gradient, some second year's growth of sweet clover was cut from various locations about the Soils Building at the Wisconsin station. The entire tops of the various lots gave the following pH values: 7.50, 7.51, 7.54, 7.55, 7.59, 7.80, and 7.90. It is therefore evident that for plants like sweet clover, it is important to compare results from corresponding parts of plants. The different actual acidities of the different parts of sweet clover, together with the different proportions of leaves, stems and petioles in the various lots, undoubtedly have been largely responsible for the above wide range of 7.50–7.90 in the pH value.

THE ACTUAL REACTION OF THE JUICE FROM THE SUCCEEDING YEAR'S GROWTH OF THE SAME CROP

When the pH values, in table 5 are compared for the first and second year's growth of sweet clover, it is found that the different parts of the first crop of the first year's growth were somewhat more acid than the second year's growth. Further experiments are necessary before a conclusion can be reached in this regard.

Since the second year's growth was much more vigorous than that of the first year, the question arises: do the more vigorously growing plants of a species show a decrease in the actual reaction of their juice compared with that of less actively growing plants of the same species?

TABLE 5

Actual reaction of the juice from different portions of sweet clover plants

ACTUAL ACIDITY	DESCRIPTION OF MATERIAL USED
First crop; First year's growth	
pH	
6.53, 6.65, 6.68, 7.38	Each lot composed of leaves, petioles and buds
6.38, 6.46, 6.48, 6.48	Each lot composed of stems only
First crop; Second year's growth	
8.00	Upper 3 inches of the tops, stem, leaves and buds
7.04	Leaves and petioles of remainder of tops (no stems)
6.68	Stems to about 2 inches above the soil (no leaves)
6.46	2 inches of lower part of stem
	2 inches of upper part of root
5.82	Root: 6-inch portion below the upper 2 inches of root

ACID RESERVE OF THE JUICE OF PLANTS GROWING IN SAME ENVIRONMENT

Since it has been observed that the juice of the second year's growth of sweet clover may be markedly alkaline in reaction, it may be of interest to compare the acid reserve of the juice from the tops of the second year's growth of sweet clover with that of the second year's growth of medium red clover. Both species were growing together in a plot, an aqueous extract of which gave a reaction of pH = 7.51. The plants were cut May 23.

Determinations were also made of the acid reserve of the root juice of the two species of plants which were obtained from the same part of the plot 3 days later. Ten-cubic-centimeter samples of juice centrifuged for 15 minutes were used in every case. The titrations were repeated on a second series of aliquots of the same juice and the results checked very closely. The results are shown in figure 7.

The curves indicate that the juice of the roots in the two cases has a greater acid reserve than the juice of the tops of similar plants cut 3 days previously. They indicate also that the juice of the medium red clover tops and of the roots has a greater acid reserve than that of the sweet clover tops and roots, respectively. Furthermore, the actual acidity of the juice of medium red clover tops is greater than that of sweet clover tops, whereas the actual acidity of the juice of the roots of both species was practically the same.

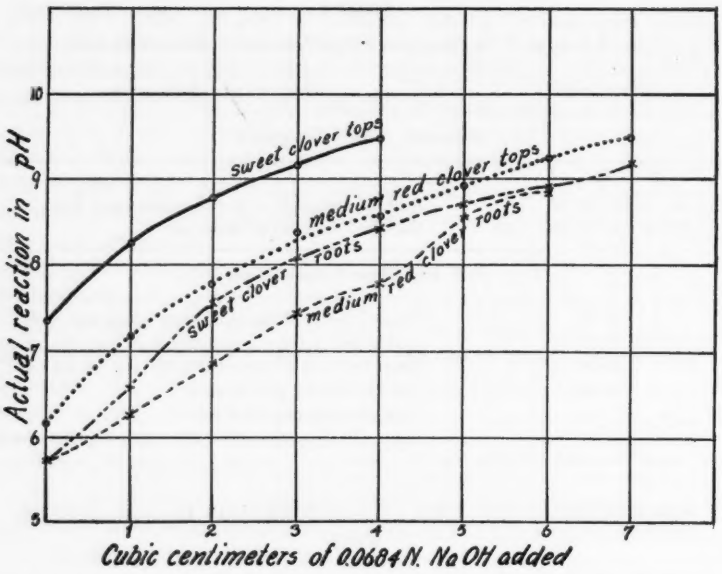

FIG. 7. CURVES SHOWING THE ACID RESERVE OF TOPS OF THE SECOND YEAR'S GROWTH OF SWEET CLOVER AND OF MEDIUM RED CLOVER CUT ON THE SAME DAY, AND THAT OF THE ROOTS OF BOTH SPECIES OBTAINED 3 DAYS LATER

THE ALKALI RESERVE OF PLANT JUICE

It was considered worth while to determine the degree to which the juice of the second year's growth of sweet clover could become alkaline. A group of such plants 24 to 34 inches high was growing luxuriantly near the edge of a burned refuse pile. The reaction of the soil extract was pH = 7.68. The uppermost 2 inches of a large number of the plants were plucked and only the very tip of the axis, together with its unfolding leaves, was retained for the determination of the actual reaction of the juice.

When the tips were cut the sun had been shining several hours, although the previous two days it had been raining. The juice was centrifuged 5 minutes and the actual reaction was found to be pH = 8.47. Since phenolphthalein turns pink at pH = 8.31, the juice of the tips of sweet clover had an actual reaction apparently alkaline to phenolphthalein. The alkali reserve of a 10-cc. portion of centrifuged juice of the tips of sweet clover was then determined by adding different amounts of standard acid to the juice and observing the new pH after each addition of the acid.[5] The values obtained in this way are given in the form of a curve in figure 8.

[5] By alkali reserve we mean the change in the actual reaction of the juice upon the addition of acid.

Since sweet clover juice was decidedly alkaline and had a high alkaline reserve, it appeared probable that it might contain more carbonates than the more acid juice of medium red clover tops. The apparatus of Van Slyke (26) was used for the estimation of carbon dioxide in the juices.

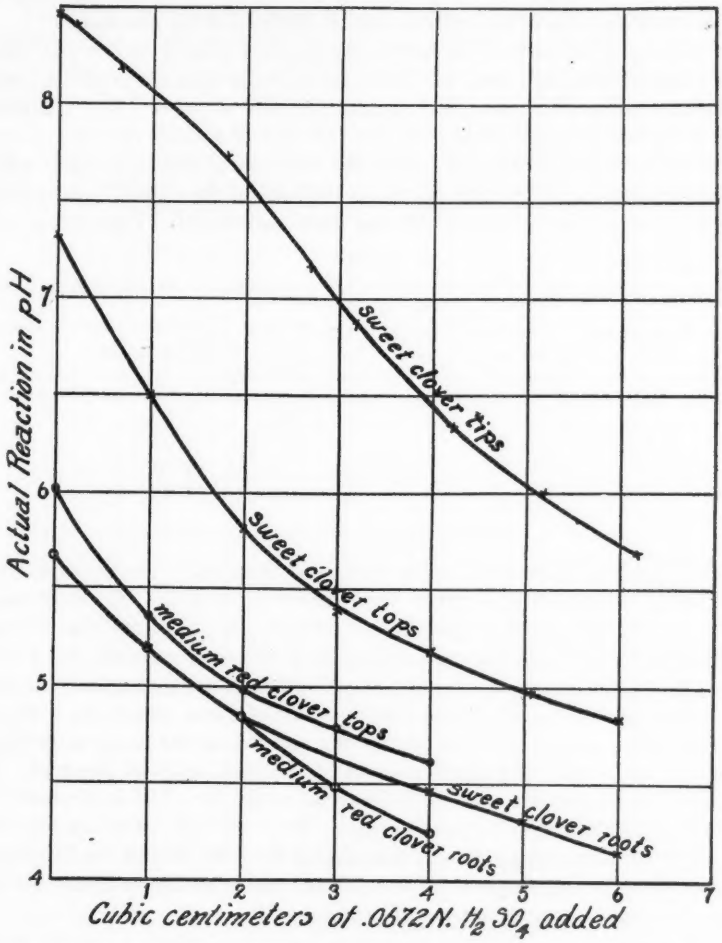

FIG. 8. CURVES SHOWING THE ALKALI RESERVE OF DIFFERENT PARTS OF SWEET CLOVER AND MEDIUM RED CLOVER

The readings of table 6 are not intended for absolute values, and hence corrections have not been made. The juice of sweet clover tops contained approximately 50 per cent more carbon dioxide than did that of medium red

clover. Undoubtedly some of the carbon-dioxide gas existed as dissolved gas in the juice, and hence without further experiments it is impossible to say how much was in the carbonate or bicarbonate form. It is for this reason that the results for medium red clover are given as a control. The results for the two species may therefore serve as a rough comparison of the relative amounts of carbonates and bicarbonates in the juice of the two species.

The juice of the roots of the second year's growth of sweet clover was then examined for its alkali reserve. The juice from the tops of the plants, from which the roots were taken, had an actual acidity of pH = 7.90. Likewise the actual reaction and alkali reserve of the juice of medium red clover roots from the same plot as that from which the sweet clover roots were taken were then determined. The alkali reserve of the juice of the tops of both species from the same plot as before also was then determined. Ten-cubic-centi-

TABLE 6

Total amount of carbon dixoide (uncorrected) in 3-cc. aliquots of the uncentrifuged juice of tops

SWEET CLOVER	MEDIUM RED CLOVER
cc.	*cc.*
0.142	
0.140	0.090
0.170	0.090
0.140	0.100
0.180	

meter samples of juice were used in the several titrations. The actual acidity and alkali-reserve determinations were carried on at about the same time, since it has been shown especially for some of the succulents that a considerable diurnal variation may take place in the reaction of the juice (14, 15, 18, 19 and 22). From the curves (fig. 8) plotted from the results obtained, it is seen that the juice of the unfolding tips of sweet clover has a much greater alkali reserve than that of the tops, and that of the tops greater than that of the roots. The alkali reserves of the root juices of sweet and of medium red clover are practically identical until pH = 4.85 is reached, at which point the curves begin to diverge. The alkali reserve of the juice of medium red clover tops is greater than that of the roots, though the difference is not as marked as with sweet clover. Much more data are necessary before any far-reaching conclusions can be drawn.

EFFECT OF THE ABSENCE OF CHLOROPHYLL (INHERITABLE) UPON THE REACTION OF PLANT JUICE

In studies upon normal and etiolated lupine seedlings Hempel (11) found that light decreases the total acidity to litmus, but increases the total acidity to phenolphthalein when compared with the total acidity of plants kept

in the dark, although the actual acidity remains nearly constant. The writer was able to secure corn seed that produced seedlings, about 25 per cent of which were pure white.[6] The juice of both the green and the colorless plants was tested for its actual and total acidity.

A preliminary experiment was carried on, using other corn seedlings. The results of the preliminary experiment are not to be emphasized on account of the fact that it is uncertain whether or not all of the seedlings received similar conditions of illumination.

The seedlings, of which only the tops were used, were 18 days old when the preliminary experiment was carried on. The actual and total acidities of 6-cc. samples of juice that had been centrifuged 15 minutes were determined. If the curves in figure 9 are examined it is found that the juice of

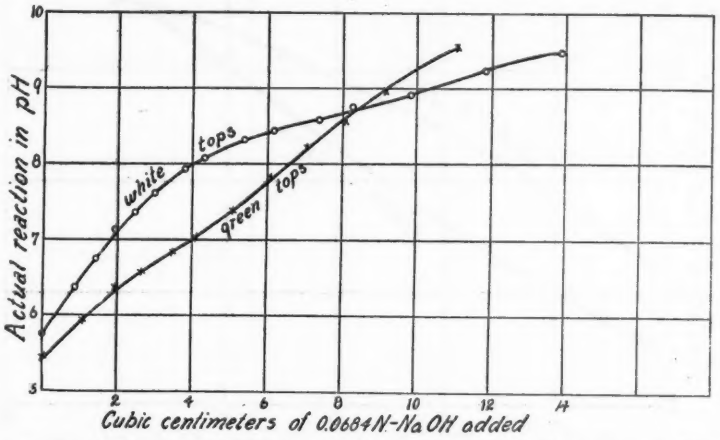

FIG. 9. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF WHITE TOPS OF CORN SEEDLINGS

the chlorophylless tops did not behave in a manner similar to that of the chlorophyllous tops as regards total acidity. The total acidity of the juice of the green tops is much greater than that of the white tops until about the point pH = 8.7 is reached, when the curves cross each other. Moreover, the initial actual acidity of the juice of the green tops was greater than that of the white tops. If the chlorophylless tops behaved like etiolated plants, then, according to the interesting observations of Hempel (11) for etiolated lupine seedlings, it should be expected that the chlorophylless tops would have shown a greater total acidity than the chlorophyllous tops to litmus—pH = 6.81, but a smaller total acidity than the chlorophyllous tops to phenolphthalein—pH = 8.31. Neither of these two conditions hold in the curves

[6] These were furnished through the kindness of E. W. Lindstrom, of the University of Wisconsin.

given; hence, it appears from this preliminary experiment, that as regards total acidity the chlorophylless plants do not behave as though they were etiolated.

Another similar experiment was carried out in which the different seedlings were grown among one another for 10 days and were given similar environmental conditions. The titrations were made on 10-cc. samples of juice that had been centrifuged 10 minutes.

The results of the determinations are given as curves in figure 10. The curves show much the same results as were observed in a preliminary experiment, and given in figure 9.

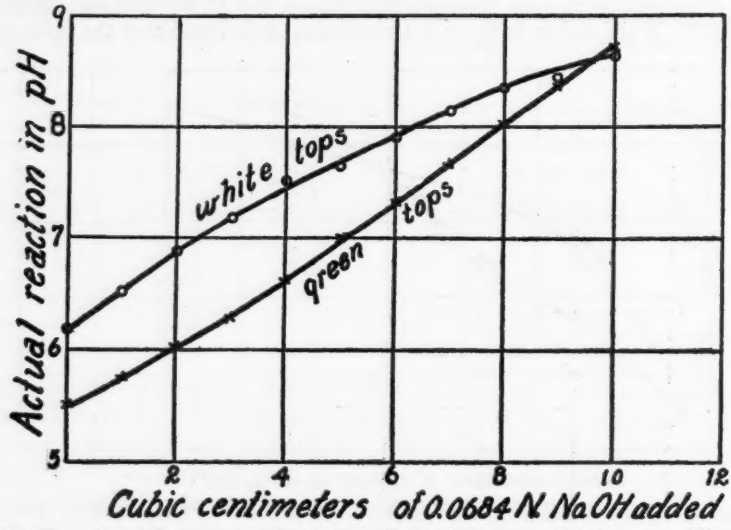

FIG. 10. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF WHITE TOPS OF CORN SEEDLINGS

Among the corn seedlings a number of the white tops were tinted somewhat green. As expected, their pH value was intermediate between that obtained for pure white and for green tops. The actual values found were as follows: green tops pH = 5.52, green-tinted white tops pH = 5.85, and white tops pH = 6.16.

EFFECT OF LIGHT AND AGE ON THE REACTION OF THE JUICE OBTAINED FROM PLANTS

Corn seed from a single ear that produced all green seedlings was planted June 3. Half of the seeded area was covered with a large box that was covered with black paper. At the end of 10 days the seedlings with green tops were 5 inches high, whereas the greenish-yellow tops of the darkened

seedlings were 9 inches high. Some of the corn seedlings with green tops were placed in a subcellar in total darkness from June 13 until June 16. Determinations were made of the actual and total acidities of the tops of both the 10- and 13-day-old plants, respectively, using in every case 10-cc. samples of juice that had been centrifuged 10 minutes.

On plotting the data obtained for the 10- and 13-day corn seedling tops (fig. 11), it is found that the total acidity of the juice of the 13-day green tops was greater than that of the 10-day green tops for any pH taken as the end-point. This is in accord with the results of Hempel (11) on white lupine seedlings in which she found that for normal seedlings the total acidity of the juice (taking litmus pH = 6.81 and phenolphthalein pH = 8.31 as end-

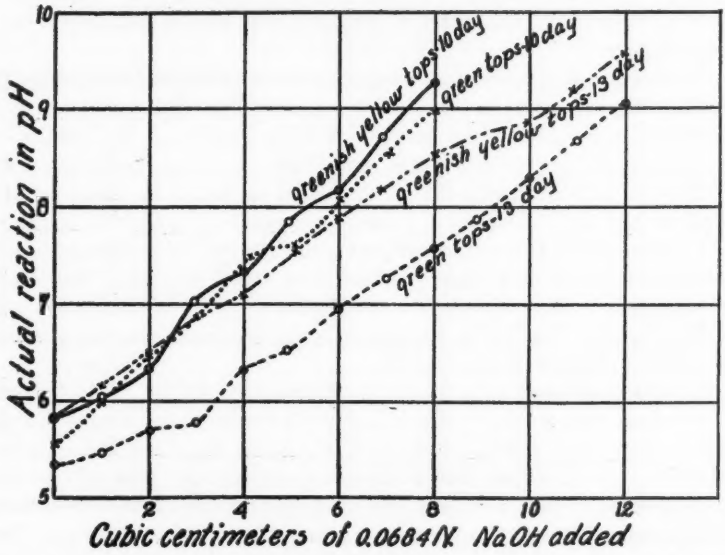

FIG. 11. CURVES SHOWING THE ACTUAL AND TOTAL ACIDITIES OF GREEN AND OF GREENISH-YELLOW TOPS OF CORN SEEDLINGS

points) increased with the increasing age of the seedlings. Undoubtedly the 10-day darkened tops must have received very faint illumination, for otherwise the tops would have been more blanched.

For the 10-day seedlings, the initial actual acidity of the darkened tops is less than that of the undarkened tops. The curves for the total acidity of the juices of the two kinds of 10-day-old tops lie very close to one another, but show to a less marked degree the same results as the 13-day-old tops. The close approach to each other of the curves for the 10-day tops may be the result of an insufficient degree of etiolation or may be due to the young age of the seedlings.

When the green seedlings were permitted to remain 10 days in the light and part were then placed in total darkness for 3 days, it is found as before that the initial actual reaction of the juice of the tops exposed 13 days to the light was greater than that of the tops exposed 10 days to the light and 3 days to total darkness. The total acidity of the juice of the tops darkened for 3 days is much less than that of the undarkened tops.

SUMMARY

The actual and total acidities and alkali reserve of a number of agricultural plants have been determined for certain conditions of growth. In order to ascertain the actual reaction of minute quantities of plant juice, a hydrogen-electrode vessel has been used that gave excellent results with only 3 to 4 drops of juice.

The reaction of the juice of a plant appears to be affected by changes in illumination, soil solution, age, and other conditions, indicating that the reaction of plant juice is the resultant of many processes. The results of preliminary experiments bearing upon some of these factors are herein reported.

The juice of buckwheat seedlings was found to possess an actual acidity of pH = 5.48 to 5.97, whereas in the mature condition a reaction of pH = 4.82 was reached. It appears that during the life cycle of buckwheat plants the juice may undergo a marked change in its actual reaction. The degree of maturity of a plant seems to be an important factor in the degree of actual acidity attained. Table 4 shows that the actual reaction of the juice of many agricultural plants is distinctly acid.

In order to obtain a measure of the buffer action of plant juice, determinations have been made of the acid and alkali reserve of the juice of certain plants. The alkali reserve of the roots of medium red clover and of sweet clover (fig. 8) is less than that of the corresponding tops. The acid reserve of the roots of medium red clover (fig. 4) is less than that of the leaves, even though the actual acidity of the roots is greater than that of the leaves. The unique case of a single plant having juice with a markedly alkaline actual reaction and a steep gradient in actual reaction is reported. The variations in the actual reaction of plant juice in the various portions of the plant have been investigated.

Although the determinations of the total acidity (table 4) have been too few in number, those reported, together with further observations to be presented later, indicate the existence of a close relation between the actual and the total acidity, and appear to show that the total acidity tends to fluctuate in the same direction as the actual acidity, although exceptions may frequently occur. It has been pointed out that the proportion of stems, leaves, petioles, etc., on plants, may influence the values obtained for the actual and the total acidities of the juice. The juice of the younger portions of a plant usually has a lower actual acidity than the older portions.

The total acidity of the juice of chlorophylless (inheritable) corn seedling tops is less than that of chlorophyllous tops until about pH = 8.6 is passed, after which the reverse is true. The actual and the total acidity of the juice of corn seedling tops increases with the increasing age of the seedlings and decreases with a reduction of the normal illumination.

There is considerable experimental data in favor of the suggestion of Truog that the main specific harmful influence of soil acidity on certain plants is due to its influence in preventing the plants from securing rapidly enough the bases that are needed to neutralize and precipitate acids within the plant. Further experiments under controlled conditions will be necessary before all the factors can be fully understood.

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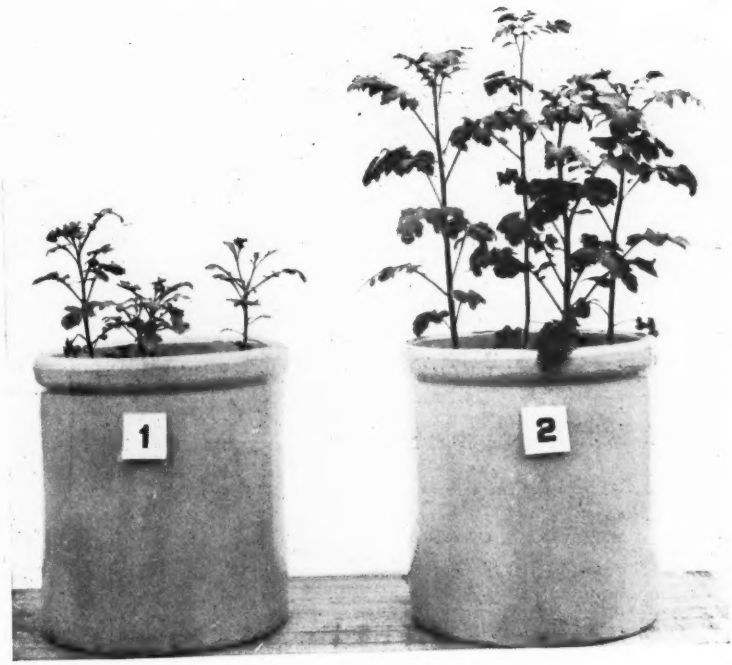
PLATE 1

EFFECT ON THE GROWTH OF WHITE MUSTARD PLANTS OF THE ADDITION OF LIME TO PLAINFIELD SAND: 1, LIMED; 2, NOT LIMED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FIELD EXPERIMENTS ON THE AVAILABILITY OF NITROGENOUS FERTILIZERS, 1908–1917[1]

J. G. LIPMAN AND A. W. BLAIR

New Jersey Agricultural Experiment Station

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In 1908 field experiments were started for the purpose of studying the relative availability of different nitrogenous fertilizer materials. The plan was developed somewhat along the lines followed for the cylinder experiments which had been started ten years before.

In the field experiments a larger number of nitrogenous materials was included and certain other variations were introduced. Lime was used on one section of the nitrogen-treated plots; another section of the plots with parallel nitrogen treatment was left without lime. The plan also provided for the study of denitrification problems.

The results of this work for the first 5 years have been published (2) and a brief summary of the second 5 years' work, so far as it relates to the lime treatment, also has appeared (1).

PLAN OF THE EXPERIMENT

For convenience the plan of the experiment may be briefly restated.

The plots are 1/20 acre in size, being separated by a 4-foot space, with a 10-foot roadway separating the two sections. The soil is a loam or gravelly loam and had not been under cultivation for some years previous to 1908.

Twenty of the plots designated as 1A to 20A have received no lime treatment during the ten years and 20 others designated as 1B to 20B received one ton of ground limestone per acre just before planting the corn in 1908, and a second application of 2 tons per acre preceding the corn crop of 1913.

Certain plots in each section receive no fertilizer, others receive one element only, others a combination of the two elements—phosphorus and potassium —and still others the three elements—nitrogen, phosphorus and potassium.

All nitrogen-treated plots receive annually 640 pounds of acid phosphate and 320 pounds of muriate of potash per acre. These are rather heavy applications but they are made in this way so that a deficiency of these materials may not become a limiting factor.

Stable manure has been applied to certain plots at the rate of 16 tons per acre annually and one plot in each section has received this amount of manure in addition to 320 pounds of nitrate of soda. One plot receives alfalfa hay,

[1] Technical Paper No. 1 of the New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

cut fine, at the rate of 2 tons per acre and another rye straw prepared in the same way and at the same rate per acre. These are used to furnish nitrogen in the form which would be furnished by crop residues or green manures. The latter however, cannot be grown on the plots where they are used, since the growing of alfalfa would complicate the nitrogen-availability studies.

The following plan gives the fertilizer treatment for each 1/20-acre plot in the two sections:

PLOT NUMBER	FERTILIZER TREATMENT
1A, 1B	Nothing
2A, 2B	16 pounds muriate of potash
3A, 3B	32 pounds acid phosphate
4A, 4B	*Minerals only
5A, 5B	Minerals, 1600 pounds cow manure
6A, 6B	Minerals, 1600 pounds horse manure
7A, 7B	Nothing
8A, 8B	Minerals, 8 pounds $NaNO_3$
9A, 9B	Minerals, 16 pounds $NaNO_3$
10A, 10B	Minerals, $Ca(NO_3)_2$ equivalent to 16 pounds $NaNO_3$
11A, 11B	Minerals, $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$
12A, 12B	Minerals, $CaCN_2$ equivalent to 16 pounds $NaNO_3$
13A, 13B	Minerals, dried blood equivalent to 16 pounds $NaNO_3$
14A, 14B	Minerals, fish equivalent to 16 pounds $NaNO_3$
15A, 15B	Minerals, concentrated tankage equivalent to 16 pounds $NaNO_3$
16A, 16B	Minerals, 200 pounds alfalfa hay
17A, 17B	Minerals, 200 pounds wheat or rye straw
18A, 18B	Minerals, 1600 pounds cow manure and 16 pounds $NaNO_3$
19A, 19B	Minerals only
20A, 20B	Minerals, 200 pounds wheat or rye straw and 16 pounds $NaNO_3$

* Minerals—32 pounds acid phosphate and 16 pounds muriate of potash

Fertilizers and manures are applied annually about the time of seeding the crops, with the exception that in the case of fall-sown crops, one-fourth of the mineral nitrogenous fertilizer is applied at the time of seeding and three-fourths as a top-dressing the following spring.

The results for the second 5-year period may be discussed in connection with the results for the first 5 years under the following headings:

(a) Yields of dry matter.
(b) Total yield of nitrogen.
(c) Percentage of nitrogen in the dry matter.
(d) Percentage of nitrogen recovered.
(e) Income and outgo of nitrogen.

YIELD OF DRY MATTER

The yields of dry matter—grain and straw or other roughage—for the 5 years, are shown in table 1.

It will be noted that this table is divided into two sections; the first constituting plots 1A to 20A (the unlimed section) and the second constituting plots 1B to 20B (the limed section). The results are reported in pounds per acre.

Plots 1 and 7 in each section have received no fertilizers. Plots 2 and 3 have received muriate of potash and acid phosphate, respectively, and plots 4 and 19 have received a combination of the two. These plots (4 and 19), therefore, constitute the checks, having received the minerals the same as the other plots, but no nitrogen.

An examination of the table shows that plot 1A has produced much better crops than plot 7A although neither receives any fertilizer. It may also be pointed out in this connection, that plots 2A and 3A have yielded more than the average shown for the check plots. This, taken in connection with the data for plot 7A, seems to indicate that the part of the field where these plots lie is better than the remainder of the section. There is at this point a slight depression and no doubt these plots have received some washings from the slightly higher points. However, so far as the nitrogenous fertilizer treatments are concerned, these plots may be eliminated from the discussion, as may also plot 7.

The influence of the nitrogenous fertilizers

The maximum yields in each section were secured on plot 18 which receives the 16 tons of manure and 320 pounds of nitrate of soda per acre. The yields from this plot are followed closely by plots 5, 6 and 20 in the two sections. It must be remembered, however, that on all of these plots excessive amounts of nitrogen have been used and it does not necessarily follow that the crops which they have yielded have been the most profitable. Indeed, in a number of cases, the excessive amount of nitrogen has caused lodging of the grain so that there was much loss at the time of harvesting. It is evident, also, that there has been a large loss of nitrogen from the soil on these plots. These points will be further discussed under the subject of "Income and Outgo of Nitrogen."

Plot 8 in each section receives one-half the standard nitrogen application, and it will be noted that the total yield on 8A is distinctly below the yields from plots that receive the full nitrogen application. The total yield on 8B, however, does not fall very much behind the total for those plots that receive the full nitrogen application. It would appear that for the crops grown the 160-pound application is not quite sufficient for a maximum yield.

The full application of nitrate of soda on plot 9A gave a total grain yield of 4065 pounds as against 3410 pounds for nitrate of lime, 3380 pounds for

TABLE 1

Yield of dry matter for the 5 years 1913–1917

PLOT NUMBER	CORN, 1913			OATS, 1914°		WHEAT, 1915		TIMOTHY		TOTAL GRAIN	TOTAL HAY, STRAW, ETC.	INCREASE OVER CHECK	
	Grain	Stalks	Cobs	Grain	Straw	Grain	Straw	1916	1917			Grain	Hay, straw, stalks, etc.
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1A	850	1,500	175	540	760	940	1,160	1,920	1,380	2,330	6,895		
2A	800	1,550	150	660	780	760	1,080	2,080	1,120	2,220	6,760		
3A	700	1,600	100	800	880	980	1,220	1,800	1,080	2,480	6,680		
4A	650	1,775	75	740	940	940	1,200	1,580	900	2,330†	6,470†		
5A	2,200	2,525	325	720	1,660	1,820	3,160	4,160	4,560	4,740	16,390	2,592	10,332
6A	2,250	2,950	325	680	1,740	1,880	3,760	4,620	4,500	4,810	17,895	2,662	11,837
7A	400	875	75	480	400	280	400	640	400	1,160	2,790		
8A	875	1,525	100	700	980	1,120	1,500	2,560	2,060	2,695	8,725	547	2,667
9A	1,625	1,975	225	700	1,200	1,740	2,520	3,880	3,880	4,065	13,680	1,917	7,622
10A	1,250	1,625	200	620	1,200	1,540	2,400	3,700	4,040	3,410	13,165	1,262	7,107
11A	1,300	1,650	175	800	1,400	1,280	1,800	1,600 880‡	1,920	3,380	9,425	1,232	3,367
12A	1,450	2,050	200	760	1,080	1,460	2,120	2,640	2,500	3,670	10,590	1,522	4,532
13A	1,325	1,950	225	560	1,160	1,420	2,160	2,800	2,400	3,305	10,695	1,157	4,637
14A	1,625	2,175	250	560	720	1,520	2,280	2,840	3,640	3,705	11,905	1,557	5,847
15A	1,750	2,250	225	340	880	1,300	1,520	2,440	2,520	3,390	9,835	1,242	3,777
16A	2,000	2,400	275	500	1,120	1,540	2,320	3,300	3,440	4,040	12,855	1,892	6,797
17A	1,075	2,000	125	460	640	800	1,080	2,360	1,480	2,335	7,685	187	1,627
18A	2,600	2,975	425	600	1,520	2,360	3,960	4,480	4,220	5,560	17,580	3,412	11,522
19A	625	1,800	125	600	740	740	800	1,420	760	1,965†	5,645†		
20A	1,950	2,400	250	660	1,200	1,740	2,680	4,240	4,440	4,350	15,210	2,202	9,152
Average	1,365	1,978	201	624	1,050	1,308	1,956	2,797	2,562	3,297	10,544		

1B	725	1,375	150	580	620	680	840	1,400	700	1,985	5,085		
2B	1,250	1,425	175	560	720	720	940	1,680	800	2,530	5,740		
3B	925	1,550	225	540	680	680	760	1,880	920	2,145	6,015		
4B	1,550	2,350	175	340	640	620	800	1,780	1,100	2,510†	6,845†		
5B	2,175	2,800	325	Sample lost		1,520	2,640	3,300	3,880	3,695	12,945	1,237	5,505
6B	1,650	1,725	250	460	1,400	1,680	3,160	3,280	3,500	3,790	13,315	1,332	5,875
7B	1,575	1,875	275	220	720	680	960	2,000	840	2,475	6,670	17	
8B	2,025	2,100	300	660	1,080	1,100	1,560	2,840	2,000	3,785	9,880	1,327	2,440
9B	1,975	2,300	350	300	1,080	1,280	1,880	3,480	3,060	3,555	12,150	1,097	4,710
10B	2,375	2,650	325	480	1,340	1,660	2,400	4,200	3,480	4,515	14,395	2,057	6,955
11B	2,550	2,725	375	500	1,260	1,600	2,480	4,080	3,540	4,650	14,460	2,192	7,020
12B	2,225	2,850	325	400	1,280	1,500	1,960	3,500	2,200	4,125	12,115	1,667	4,675
13B	2,225	2,700	325	580	1,120	1,400	2,140	3,420	2,240	4,205	11,945	1,747	4,505
14B	2,250	2,675	325	520	1,080	1,260	1,880	3,000	2,140	4,030	11,100	1,572	3,660
15B	2,350	2,425	350	520	980	1,080	1,720	3,040	2,200	3,950	10,715	1,492	3,275
16B	2,200	2,575	350	380	1,180	1,340	2,420	3,240	3,120	3,920	12,885	1,462	5,445
17B	2,600	2,525	375	520	660	1,140	1,400	2,900	3,220	4,260	11,080	1,802	3,640
18B	2,950	2,625	425	600	2,060	2,320	4,120	4,160	4,560	5,870	17,950	3,412	10,510
19B	1,025	2,300	175	480	680	900	1,160	2,000	1,720	2,405†	8,035†		
20B	2,425	2,625	400	820	1,320	1,940	2,880	4,280	4,220	5,185	15,725	2,727	8,285
Average	1,951	2,309	299	498	1,047	1,255	1,905	2,973	2,472	3,679	10,953		

* The oats crop was damaged by heavy rains at harvest time.

† Check.

‡ Second growth—largely crab grass.

sulfate of ammonia and 3670 pounds for calcium cyanamid. The full application of nitrate on 9B, on the other hand, gave a total yield of 3555 pounds of grain as against 4515 pounds for calcium nitrate, 4650 pounds for ammonium sulfate and 4125 pounds for calcium cyanamid.

Seven of the plots 9 to 15, inclusive, in each section receive equivalent amounts of nitrogen and it is of interest to compare the average yields from these plots with the average from the two check plots. These figures are shown in table 2.

In all cases the nitrogen-treated plots show a substantial increase over the check plots. In most cases this increase amounts to more than 50 per cent, which means that the outlay for nitrogen has proven a good investment. It is quite possible, however, that had the application of nitrate been less than 320 pounds—200 or 250 pounds per acre—the increase in yield might have been as great, which would have meant a larger profit than was obtained with the 320 pounds.

TABLE 2

Average yield of dry matter—check plots compared with nitrogen-treated plots

		UNLIMED SECTION		LIMED SECTION	
		1908–1912	1913–1917	1908–1912	1913–1917
		lbs.	*lbs.*	*lbs.*	*lbs.*
Check	Grain	4,544.4	2,147.5	3,907 5	2,457.5
	Straw, etc	9,044.8	6,057.5	8,818.7	7.440.0
Seven nitrogen treated plots	Grain	5,831.2	3,560.7	6,248.9	4,147 1
	Straw, etc	14,179.4	11,328.0	14,275.6	12,411.4

It will be noted that the average yields for the second 5-year period are less in all cases than those for the first 5-year period. This is partly due to the fact that a residual crop of corn was grown after the oat crop of 1909. Such residual crop was not grown in the second 5-year period.

Four of the seven plots referred to above receive mineral nitrogenous materials and three receive organic materials in equivalent amounts. We may, therefore, compare the yields obtained from plots receiving the organic materials with those from plots receiving the mineral materials. This comparison is made for both sections and for the two 5-year periods in table 3, the figures being the average for three plots in one case and for four in the other.

An examination of this table shows that in all cases, the yields of both grain and straw, stover, etc., are larger where the mineral nitrogenous materials were used than with the organic materials. This is in agreement with results obtained in cylinder experiments, reference to which has already been made.

It has been thought by some, that the residual effect of organic materials would outweigh the quicker effect of readily soluble materials. It does not

appear to be so. The readily soluble materials give the plant a good start at a time when the nitrification of organic matter is slow and this good start apparently keeps the plant in the lead to the end. With such a start, it is better able to use soil moisture and the natural plant-food than is the plant which gets a slower start. Furthermore, it is very probable that during the time required for the transformation of the organic nitrogen into available plant-food the loss from this is greater than the loss by leaching from the mineral compounds.

Whatever reason may be assigned for the difference, it is very evident that a pound of nitrogen from the mineral materials is more efficient than a pound from the organic materials. This statement is made on the basis of results secured during ten years of field experiments and twenty years of cylinder experiments.

TABLE 3

Average yield of dry matter—mineral and organic nitrogenous materials compared

		UNLIMED SECTION		LIMED SECTION	
		1908–1912	1913–1917	1908–1912	1913–1917
		lbs.	*lbs.*	*lbs.*	*lbs.*
Average four mineral materials	Grain.....	6,002.5	3,631.2	6,379.6	4,211.2
	Straw, etc.....	14,686.4	11,715.0	15,534.6	13,280.0
Average three organic materials	Grain.....	5,602.9	3,466.7	6,074.5	4,061.7
	Straw, etc.....	13,503.4	10,812.0	12,596.8	11,253.3

The yields from plots 16 and 17 are of interest. It will be recalled that plot 16 received alfalfa hay (cut fine) at the rate of 2 tons per acre and plot 17 rye straw prepared in the same way and applied at the same rate per acre. Naturally, a given amount of alfalfa furnishes more nitrogen than an equal amount of the straw, and furthermore, it is in a more available form. It is not surprising therefore, to find the yields from 16A considerably larger than those from 17A. In an acid soil, the straw decomposes very slowly and therefore yields very little available nitrogen.

On the other hand, the 5-year yield from 17B is larger than the yield from 16B. This is probably due to the fact that for five or six years volunteer clover and vetch have been coming into 17B. The lime and minerals which are applied on this plot make the conditions favorable for the legumes and as a result, the latter flourish at the expense of grass and grain. On 16B the alfalfa hay on a well limed soil furnishes enough available nitrogen to encourage the growth of the grass and grain crops to the exclusion of volunteer legumes.

TABLE 4

Yield of nitrogen for the 5 years 1913–1917

PLOT NUMBER	CORN, 1913				OATS, 1914			WHEAT, 1915			TIMOTHY		NITROGEN IN		TOTAL NITROGEN
	Grain	Stalks	Cob	Total	Grain	Straw	Total	Grain	Straw	Total	1916	1917	Grain	Hay, Straw, Stalks, etc.	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1A	12.95	16.89	1.00	30.84	10.88	5.70	16.58	19.34	4.11	23.45	17.51	13.32	43.17	58.53	101.7
2A	11.30	11.16	0.83	23.29	12.78	5.70	18.48	15.78	4.46	20.24	21.44	11.47	39.86	55.06	94.9
3A	10.23	13.28	0.74	24.25	15.54	5.96	21.50	20.34	3.84	24.18	17.08	9.89	46.11	50.79	96.9
4A	8.87	13.86	0.30	23.03	14.92	5.62	20.54	18.96	4.61	23.57	14.84	8.51	42.75	47.74	90.5*
5A	34.98	20.91	1.35	57.24	17.20	15.08	32.28	35.27	13.68	48.95	31.49	35.48	87.45	117.99	205.4
6A	33.57	25.37	1.19	60.13	16.72	19.22	35.94	39.41	17.41	56.82	37.93	36.36	89.70	137.48	227.2
7A	6.08	7.08	0.53	13.69	8.82	3.06	11.88	5.84	1.50	7.34	6.53	3.74	20.74	22.44	43.2
8A	12.01	9.32	0.44	21.77	15.04	7.70	22.74	19.94	5.18	25.12	18.69	15.02	46.99	56.35	103.3
9A	25.04	12.46	1.22	38.72	16.98	11.66	28.64	34.22	11.16	45.38	26.19	28.29	76.24	90.98	167.2
10A	19.38	16.35	1.17	36.90	14.10	10.48	24.58	30.60	10.39	40.99	25.64	27.84	64.08	91.87	156.0
11A	19.77	13.51	0.85	34.13	18.68	13.60	32.28	29.98	12.22	42.20	25.77	19.85	68.43	85.80	154.1
12A	19.49	14.56	0.97	35.02	17.34	9.14	26.48	30.62	8.56	39.18	23.13	22.40	67.45	78.76	146.2
13A	19.64	14.04	1.20	34.88	11.48	8.90	20.38	28.64	10.20	38.84	26.29	20.57	59.76	81.20	141.0
14A	27.30	16.10	1.21	44.61	12.54	6.04	18.58	28.10	10.53	38.63	24.34	28.32	67.94	86.54	154.5
15A	26.11	18.63	0.96	45.70	7.72	8.32	16.04	25.83	6.28	32.11	22.91	21.85	59.66	78.95	138.6
16A	34.58	28.42	1.07	64.07	12.30	11.08	23.38	32.56	8.68	41.24	27.98	28.10	79.44	105.33	184.8
17A	17.63	14.80	0.91	33.34	9.60	4.86	14.46	17.39	4.68	22.07	20.67	13.41	44.62	59.33	104.0
18A	43.94	27.28	1.68	72.90	14.72	15.16	29.88	53.41	18.73	72.14	38.39	41.99	112.07	143.23	255.3
19A	8.83	11.02	0.88	20.73	12.04	5.02	17.06	15.64	3.07	18.71	12.30	7.41	36.51	39.70	76.2*
20A	32.76	21.77	1.43	55.96	15.00	11.02	26.02	36.96	14.50	51.46	34.43	32.37	84.72	115.52	200.2
Average	21.22	16.34	1.00	38.56	13.72	9.17	22.89	26.94	8.69	35.63	23.68	21.26	61.88	79.66	142.0

1B	11.60	12.35	0.70	24.65	12.30	5.02	17.32	13.45	3.23	16.68	14.56	6.62	37.35	42.48	79.8
2B	18.28	14.62	0.92	33.82	11.48	5.20	16.68	15.03	3.23	18.26	17.93	7.64	44.79	49.54	94.3
3B	14.71	14.83	1.22	30.76	11.42	5.82	17.24	14.47	2.69	17.16	19.72	8.06	40.60	52.34	92.9
4B	20.49	18.54	0.78	39.81	6.88	4.56	11.44	13.37	2.99	16.36	15.41	9.54	40.74	51.82	92.6*
5B	35.24	26.52	1.35	63.11	†	†	†	30.42	10.40	40.82	31.32	32.09	65.66	101.68	167.3
6B	26.90	16.68	1.26	44.84	10.54	13.60	24.14	39.65	14.60	54.25	34.41	31.36	77.09	111.91	189.0
7B	25.20	19.05	1.14	45.39	3.94	4.76	8.70	14.07	4.63	18.70	20.24	7.28	43.21	57.10	100.3
8B	31.21	20.92	1.48	53.61	14.94	8.66	23.60	20.94	6.15	27.09	22.81	15.76	67.09	75.78	142.9
9B	31.60	20.19	1.80	53.59	7.24	11.36	18.60	26.11	7.95	34.06	28.57	23.81	64.95	93.68	158.6
10B	36.36	18.82	1.32	56.50	11.56	13.38	24.94	33.70	8.74	42.44	32.93	26.03	81.62	101.22	182.8
11B	37.79	23.93	1.70	63.42	11.94	12.80	24.74	33.28	11.24	44.52	33.86	26.83	83.01	110.36	193.4
12B	35.18	20.26	1.38	56.82	9.16	12.32	21.48	31.05	6.94	37.99	34.16	21.89	75.39	96.95	172.3
13B	34.29	21.09	1.48	56.86	13.80	10.78	24.58	28.56	7.15	35.71	28.39	19.85	76.65	88.74	165.4
14B	34.67	18.75	1.35	54.77	12.14	9.04	21.18	26.45	7.03	33.48	28.17	20.03	73.26	84.37	157.6
15B	36.92	25.34	1.35	63.61	12.46	10.30	22.76	21.83	5.75	27.58	27.18	19.71	71.21	89.63	160.8
16B	35.42	29.97	1.49	66.88	9.38	12.30	21.68	29.29	8.32	37.61	27.48	28.27	74.09	107.83	181.9
17B	44.69	25.91	1.93	72.53	10.62	4.48	15.10	24.70	5.78	30.48	25.40	37.09	80.01	100.59	180.6
18B	46.91	25.38	1.81	74.10	14.98	20.92	35.90	54.52	14.58	69.10	37.94	46.24	116.41	146.87	263.3
19B	16.68	14.51	0.94	32.13	9.92	4.62	14.54	17.40	3.54	20.94	19.52	19.99‡	44.00	52.67	96.7*
20B	41.20	25.88	1.84	68.92	19.36	12.12	31.48	42.04	11.89	53.93	34.37	35.32	102.68	121.42	224.1
Average	30.77	20.68	1.36	52.81	11.27	9.58	20.85	26.52	7.34	33.86	26.72	21.65	67.99	86.85	154.8

* Averaged for check.

† Sample lost.

‡ 9.54, the value for 4B, taken as the check and for making up total.

The lime factor

This phase of the question has been fully discussed in an earlier paper (2); and only a brief reference need be made to it here. If the averages for the two sections (limed and unlimed) are examined, it will be noted that the corn crop of 1913 shows some increase from the lime treatment. The other crops in the rotation show little or no response to lime. The total yields for the 5 years for the limed section are only a few hundred pounds in excess of the total for the unlimed section.

These figures, taken in conjunction with nitrogen determinations made on samples of soil from both sections, indicate that there has been a greater loss of nitrogen from the limed than from the unlimed plots. Or to express the thought in another way, the acid condition of the unlimed plots has tended to conserve the nitrogen supply of the soil. It would thus appear, that for soils where oxidation of the organic matter goes on rather rapidly, the stimulating effect of lime is not required in the growing of non-leguminous crops.

TOTAL YIELD OF NITROGEN

The total yield of nitrogen for the two sections is shown in table 4.

In general, the plots that yielded the largest amounts of dry matter also yielded the largest amounts of total nitrogen. The lowest total yield for the unlimed section was 43.2 pounds per acre from 7A and the highest yield for this section was 255.3 pounds per acre from plot 18A. The lowest yield from the limed section was 79.8 pounds from plot 1B and the highest 263.3 pounds from 18B.

The average *annual* yield for all the unlimed plots was 28.3 pounds per acre and for all the limed plots 31.1 pounds per acre. The average annual yield for 18A was 51.1 pounds per acre and for 18B 52.7 pounds.

Remembering that these two plots receive between 200 and 225 pounds of nitrogen per acre annually, we see from the above figures that only about 25 per cent of the applied nitrogen is recovered in the crop.

A comparison of the total amount of nitrogen recovered in the crops from the seven plots which receive equivalent amounts of nitrogen is of interest. These figures for both the limed and the unlimed sections are shown in table 5.

In each section the corn and wheat have drawn most heavily upon the nitrogen. The oat crop was unusually small and therefore the amount of nitrogen recovered in this crop is low. The nitrogen taken out by the timothy varies from about 23 to 30 pounds per acre annually. The corn crop on section B removed on an average 57.9 pounds of nitrogen per acre which is almost as much as is contained in 400 pounds of nitrate of soda. For the unlimed section, the seven plots which receive equivalent amounts of nitrogen show an average total of 149.6 pounds per acre of nitrogen removed by the five crops. The average total amount removed by the crops on the corresponding plots of the limed section is 170.2 pounds per acre.

Here is shown a total gain of about 20 pounds in favor of the limed plots. However, reference to the figures for the corn crop of 1913 show that the gain was practically all made by this crop.

TABLE 5

Total yields of nitrogen from plots which receive nitrogenous fertilizers in equivalent amounts

PLOT NUMBER	CORN 1913	OATS 1914	WHEAT 1915	TIMOTHY 1916	TIMOTHY 1917	TOTAL
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
9A	38.7	28.6	45.4	26.2	28.3	167.2
10A	36.9	24.6	41.0	25.6	27.8	155.9
11A	34.1	32.3	42.2	25.8	19.9	154.3
12A	35.0	26.5	39.2	23.1	22.4	146.2
13A	34.9	20.4	38.8	26.3	20.6	141.0
14A	44.6	18.6	38.6	14.0	28.3	144.1
15A	45.7	16.0	32.1	22.9	21.9	138.6
Average	38.56	23.86	39.6	23.4	24.17	149.6
9B	53.6	18.6	34.1	28.6	23.8	158.7
10B	56.5	24.9	42.4	32.9	26.0	182.7
11B	63.4	24.7	44.5	33.9	26.8	193.3
12B	56.8	21.5	38.0	34.2	21.9	172.4
13B	56.9	24.6	35.7	28.4	19.9	165.5
14B	54.8	21.2	33.5	28.2	20.0	157.7
15B	63.6	22.8	27.6	27.2	19.7	160.9
Average	57.9	22.6	36.5	30.5	22.6	170.2

The last column of figures in table 5 shows that the average amount of nitrogen recovered from the four plots (9 to 12) which receive mineral nitrogenous materials exceeds the average amount recovered from the three plots (13 to 15) which receive organic nitrogenous materials.

The 5-year averages are as follows:

	UNLIMED	LIMED
	lbs.	*lbs.*
Plots 9 to 12	155.9	176.8
Plots 13 to 15	141.2	161.3

This is in accord with the figures reported in the yield of dry matter and gives further evidence that the mineral nitrogenous materials are more efficient in crop production than the organic materials.

THE PERCENTAGE OF NITROGEN IN THE DRY MATTER

The percentage of nitrogen in the dry matter of all the crops for both the limed and the unlimed sections is shown in table 6.

For a given crop these percentages do not vary greatly, and they are therefore not of so much importance as the total nitrogen, but it seems best that they should constitute a part of the record.

TABLE 6

Percentage of nitrogen in dry matter, 1913–1917

PLOT NUMBER	CORN, 1913			OATS, 1914		WHEAT, 1915		TIMOTHY HAY		AVERAGE
	Grain	Stalks	Cobs	Grain	Straw	Grain	Straw	1916	1917	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1A	1.522	1.126	0.573	2.015	0.749	2.057	0.354	0.912	0.965	1.141
2A	1.413	0.720	0.553	1.935	0.731	2.076	0.413	1.031	1.024	1.099
3A	1.462	0.830	0.741	1.943	0.678	2.076	0.315	0.949	0.916	1.101
4A	1.364	0.781	0.405	2.015	0.597	2.017	0.384	0.939	0.945	1.049
5A	1.590	0.828	0.415	2.389	0.909	1.938	0.433	0.757	0.778	1.114
6A	1.492	0.860	0.366	2.460	1.105	2.097	0.463	0.821	0.808	1.163
7A	1.521	0.809	0.711	1.836	0.767	2.087	0.374	1.021	0.936	1.118
8A	1.373	0.611	0.435	2.149	0.785	1.780	0.345	0.730	0.729	0.993
9A	1.541	0.631	0.543	2.425	0.972	1.966	0.443	0.675	0.729	1.102
10A	1.550	1.006	0.583	2.273	0.874	1.987	0.433	0.693	0.689	1.121
11A	1.521	0.819	0.484	2.336	0.972	2.342	0.679	1.094*	1.034	1.253
12A	1.344	0.710	0.484	2.282	0.847	2.097	0.404	0.876	0.896	1.104
13A	1.482	0.720	0.534	2.051	0.767	2.017	0.472	0.939	0.857	1.093
14A	1.680	0.740	0.484	2.238	0.838	1.849	0.462	0.857	0.778	1.103
15A	1.492	0.828	0.425	2.273	0.945	1.987	0.413	0.939	0.867	1.130
16A	1.729	1.184	0.445	2.461	0.990	2.114	0.374	0.848	0.817	1.218
17A	1.640	0.740	0.731	2.086	0.758	2.174	0.433	0.876	0.906	1.149
18A	1.690	0.917	0.395	2.452	0.998	2.263	0.473	0.857	0.995	1.227
19A	1.413	0.612	0.701	2.006	0.678	2.114	0.384	0.866	0.975	1.083
20A	1.680	0.907	0.573	2.273	0.918	2.124	0.541	0.812	0.729	1.173
Average	1.525	0.819	0.529	2.195	0.844	2.008	0.430	0.875	0.869	1.127
1B	1.600	0.898	0.464	2.122	0.811	1.978	0.384	1.040	0.945	1.138
2B	1.462	1.026	0.524	2.051	0.722	2.087	0.344	1.067	0.955	1.138
3B	1.590	0.957	0.543	2.113	0.856	2.128	0.354	1.049	0.876	1.163
4B	1.413	0.789	0.445	2.024	0.713	2.157	0.374	0.866	0.867	1.072
5B	1.620	0.947	0.415	lost	lost	2.001	0.394	0.949	0.827	1.022
6B	1.630	0.967	0.504	2.291	0.972	2.360	0.462	1.049	0.896	1.237
7B	1.600	1.016	0.415	1.792	0.660	2.069	0.482	1.012	0.867	1.101
8B	1.541	0.996	0.494	2.264	0.802	1.904	0.394	0.803	0.788	1.109
9B	1.600	0.878	0.514	2.416	1.052	2.040	0.423	0.821	0.778	1.169
10B	1.531	0.710	0.405	2.408	0.998	2.030	0.364	0.784	0.748	1.109
11B	1.482	0.878	0.445	2.389	1.016	2.080	0.453	0.830	0.758	1.148
12B	1.581	0.711	0.425	2.291	0.963	2.070	0.354	0.976	0.995	1.152
13B	1.541	0.781	0.454	2.380	0.963	2.040	0.334	0.830	0.886	1.134
14B	1.541	0.701	0.415	2.336	0.838	2.099	0.374	0.939	0.936	1.131
15B	1.571	1.045	0.385	2.398	1.052	2.021	0.334	0.894	0.896	1.177
16B	1.610	1.164	0.425	2.469	1.043	2.186	0.344	0.848	0.906	1.222
17B	1.719	1.026	0.514	2.042	0.678	2.167	0.413	0.876	1.152	1.176
18B	1.590	0.967	0.425	2.496	1.016	2.350	0.354	0.912	1.014	1.236
19B	1.334	0.631	0.534	2.068	0.678	1.933	0.305	0.976	1.162	1.069
20B	1.699	0.986	0.435	2.362	0.918	2.167	0.413	0.803	0.837	1.180
Average	1.512	0.904	0.459	2.248	0.882	2.093	0.383	0.916	0.904	1.144

* A second growth—largely crab-grass—gave 0.94 per cent nitrogen.

It may be noted in passing that the corn grain contains about 1.5 per cent of nitrogen, the oats and wheat grain a little over 2 per cent and the timothy hay about 0.9 per cent. The oats straw contains about twice as much nitrogen as the wheat straw.

Attention may be called to the rather high average percentage for plots 11A, 16A, 16B, 18A and 18B. The condition of the soil on 11A is abnormal and this no doubt accounts for the high percentage of nitrogen in the crop from this plot. It has sometimes been observed that a soil very deficient in nitrogen produces a small crop having an exceptionally high nitrogen content. The other plots mentioned receive rather heavy applications of nitrogenous fertilizers, and this excess of nitrogen has influenced the nitrogen content of the crop.

It is well known, for example, that in pot experiments the normal nitrogen content of the plant may be more than doubled by heavy applications of nitrate of soda. It has also been found that the nitrogen content of leguminous plants grown on limed land is generally higher than that of the same plants grown on land that is deficient in lime.

It will be observed that there is little difference between the nitrogen content of the crops of this rotation (non-legumes) whether grown on the limed or the unlimed section.

THE PERCENTAGE OF NITROGEN RECOVERED

The check plot is supposed to show the amount of nitrogen which the crop gets from the natural supply of nitrogen that is found in the soil. Then the difference between this amount and the amount recovered through the crop receiving a nitrogenous fertilizer should show the amount of the applied nitrogen which that crop was able to win back, or recover. From this figure the percentage of applied nitrogen may be calculated.

The percentage recovery for the various nitrogenous materials has been thus calculated for the 5-year period and is shown in table 7.

Attention may be drawn to the low average recovery from plots 5, 6 and 18. All these plots receive annual applications of manure at the rate of 16 tons per acre and in addition plot 18 receives 320 pounds of nitrate of soda per acre. Less than one-sixth of the applied nitrogen is won back in the crops. There is, therefore, a heavy loss of nitrogen from these plots. No doubt much of this loss is due to the escaping of nitrogen gas and ammonia while decomposition is taking place. So far, no direct means has been found of measuring such losses.

Analysis shows that these soils have made slight gains in nitrogen content since the work was started in 1908. But as Russell (3) points out, such gains cannot go on indefinitely. There comes a time, he says, when "a point of equilibrium is reached, higher or lower according to the soil conditions, where further gains are balanced by losses, so that the nitrogen content remains

constant." It would appear from the heavy loss sustained by these plots that the point of equilibrium has been reached and that the gains are balanced by the losses. The fact that these plots are receiving excessive amounts of nitrogen is attested by the serious lodging of the grain.

TABLE 7

Percentage of nitrogen recovered in the different crops

PLOT NUMBER	CORN, 1913	OATS, 1914	WHEAT, 1915	TIMOTHY HAY		5-YEAR AVERAGE
				1916	1917	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
5A	20.38	7.98	18.25	11.22	15.24	14.61
6A	17.72	7.54	17.53	18.77	19.25	16.16
8A	Loss	15.72	15.87	20.91	29.22	16.34
9A	34.39	19.62	48.34	25.77	42.07	34.04
10A	30.63	11.52	39.58	24.65	41.14	29.50
11A	25.01	26.88	42.00	24.91	24.61	28.68
12A	26.80	15.31	35.97	19.52	29.88	25.50
13A	26.53	3.15	35.30	25.98	26.09	23.41
14A	46.39	Loss	34.88	22.00	42.14	29.08
15A	48.61	Loss	21.87	19.07	28.75	23.66
16A	28.70	4.57	19.70	14.41	20.79	17.63
17A	12.45	Loss	2.71	35.50	13.60	12.85
18A	22.94	5.05	25.19	11.70	14.87	15.95
20A	17.07	11.02	35.74	30.25	27.30	24.28
Average	25.54	9.17	28.07	21.76	26.78	22.26
5B	15.64	Sample lost	14.55	8.67	12.49	12.84*
6B	4.11	5.02	17.49	13.05	14.79	10.89
8B	71.98	42.28	33.66	21.81	25.74	39.09
9B	35.97	11.16	30.73	22.87	29.53	26.05
10B	41.88	23.81	47.44	31.57	34.12	35.76
11B	55.94	23.41	51.59	33.47	35.78	40.04
12B	42.55	16.91	38.57	34.09	25.56	31.53
13B	42.61	23.49	34.02	22.30	21.33	28.75
14B	38.38	16.31	29.57	21.85	21.71	25.56
15B	56.41	19.46	17.81	19.89	21.05	26.92
16B	20.99	8.66	18.58	10.01	19.33	15.51
17B	39.69	13.67	34.11	39.65	68.70	39.16
18B	17.14	10.45	24.91	9.81	16.04	15.67
20B	23.36	28.21	41.59	24.51	28.84	29.30
Average	36.19	18.68	31.04	22.40	26.80	26.93

* 12.84 is 4-year average.

Note—Plots 1, 2, 3, 4, 7, and 19 received no nitrogen.

It is of interest to compare the percentage of nitrogen recovered from the four mineral nitrogenous plots with the recovery from the three plots which receive organic materials. The averages for these plots for the two 5-year periods and for the limed and the unlimed section are shown in table 8.

Throughout the 10 years and for both sections the mineral materials have given the highest percentage recovery.

The differences are not so great, however, for the second 5-year period as for the first. This may mean some cumulative effect from the organic materials, but in the cylinder experiments referred to such cumulative effect, if it exists, has not brought the recovery from an organic material up to the level of the mineral materials during a period of 20 years.

It may be pointed out that during the second 5-year period only plots 10B and 11B of the seven under consideration show a recovery of more than one-third of the applied nitrogen.

It is exceedingly unfortunate that so much of this most expensive element must be lost. The situation should stimulate soil investigators everywhere to put forth efforts to find a means of stopping some of this loss. Furthermore it should be a warning to the farmer to secure a greater proportion of his nitrogen from the atmosphere through means of leguminous crops. If he must lose two-thirds or more of his purchased nitrogen then it will be economy to purchase only such amounts as can be used most effectively.

TABLE 8

Per cent of nitrogen recovered from mineral nitrogenous materials as compared with organic materials

	UNLIMED SECTION		LIMED SECTION	
	1908–1912	1913–1917	1908–1912	1913–1917
	per cent	*per cent*	*per cent*	*per cent*
Average, 4 mineral materials	40.1	29.4	48.5	33.3
Average, 3 organic materials	27.3	23.5	28.5	27.1

Indeed it has been shown that the percentage recovery is generally higher with small than with large applications. Thus small applications supplemented by the nitrogen secured through legumes will enable the farmer to get better results and with less cash outlay.

An examination of the table shows that less nitrogen has been recovered from 5B, 6B and 18B than from the corresponding A plots. This means that on these plots available nitrogen is not the limiting factor. Here nitrification takes place rapidly enough without the stimulating effect of carbonate of lime. Lime simply adds fuel to the fires of destruction.

Plot 8A gives a 5-year average return of 16.34 per cent, whereas 8B, the corresponding limed plot, shows a recovery of 39.09 per cent. This emphasizes the value of a small amount of available nitrogen when lime is supplied. On the unlimed section nitrate of soda (plot 9A) shows a higher average recovery than either ammonium sulfate, calcium nitrate or calcium cyanamid, but on the limed section all these show a higher recovery than the nitrate.

A 5-year average recovery of 40 per cent for 11B (the highest 5-year average) gives evidence of the value of lime in promoting nitrification in a soil that

would otherwise have been made acid by the continued use of sulfate of ammonia. In contrast 11A which is the unlimed sulfate of ammonia plot, shows a recovery of 28.68 per cent.

Plot 17B, which receives its nitrogen in the form of rye straw (a slowly available material) and is limed, shows a recovery of 39.16 per cent as against a recovery of 12.85 per cent for 17A, the corresponding unlimed plot.

The seven unlimed plots (9A to 15A) which receive equivalent amounts of nitrogen, show a 5-year average recovery of 27.7 per cent as against 30.7 per cent for the corresponding limed plots, a difference of 3 per cent in favor of the latter.

THE INCOME AND OUTGO OF NITROGEN

This question has been partially covered by the discussion under "Percentage of Nitrogen Recovered." It seems worth while, however, to record here the amount of nitrogen applied each year and the total amount recovered in the crops for the 5 years. These figures, on the acre basis, are shown in table 9.

It may be noted that plots 5, 6 and 18 receive much larger applications of nitrogen than plots 9 to 15, inclusive. Plot 18 received during the 5 years nitrogen equivalent to 1081.6 pounds per acre, which is more than four times the standard application. The percentage of nitrogen recovered from this plot has already been discussed.

The variations in the amount of nitrogen furnished by the rye-straw, plot 17, are probably due to the fact that in some cases more grain was left in the straw than in others.

Through an oversight the alfalfa hay and rye straw used on plots 16 and 17 in 1916 were not analyzed. It was therefore necessary to estimate the amount of nitrogen in these materials.

The percentage of the applied nitrogen that was recovered in the crop is shown in the last column of the table. These figures should correspond with the figures given in table 7, but on account of slight differences in decimals and irregularities in two or three of the series, there are some variations.

A study of the income and outgo of nitrogen would not be complete without a consideration of the nitrogen content of the soil. Unfortunately samples from only a few of the plots were analyzed when the work was started; however, these analyses may be taken as a fair average of the nitrogen content of the soil at that time. Analyses made in 1909 of samples from five of the plots show an average nitrogen content of 0.1118 per cent and a carbon content of 1.22 per cent. Samples were collected from all the plots at the end of the first 5-year period and again at the end of the second 5-year period (1917), and nitrogen and carbon determinations have been made on all these samples, the results of which are reported in table 10.

TABLE 9

Nitrogen applied for each crop (acre basis) and the average percentage of nitrogen recovered

	NITROGEN APPLIED, 1913	NITROGEN APPLIED, 1914	NITROGEN APPLIED, 1915	NITROGEN APPLIED, 1916	NITROGEN APPLIED, 1917	TOTAL NITROGEN APPLIED,	TOTAL NITROGEN RECOVERED IN CROPS	TOTAL INCREASE OVER CHECK	AVERAGE PERCENTAGE OF NITROGEN RECOVERED
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*
1A							101.7		
2A							94.9		
3A							96.9		
4A							90.5	Check	
5A	173.5	169.0	152.3	159.7	180.5	835.0	205.4	122.0	14.6
6A	215.8	227.2	203.5	129.7	147.5	923.7	227.2	143.8	15.6
7A							43.2		
8A	24.5	25.0	25.0	24.5	24.1	123.1	103.3	19.9	16.2
9A	49.0	50.1	50.1	49.0	48.3	246.5	167.2	83.8	34.0
10A	49.0	50.1	50.1	49.0	48.3	246.5	156.0	72.6	29.5
11A	49.0	50.1	50.1	49.0	48.3	246.5	154.1	70.7	28.7
12A	49.0	50.1	50.1	49.0	48.3	246.5	146.2	62.8	25.5
13A	49.0	50.1	50.1	49.0	48.3	246.5	141.0	57.6	23.4
14A	49.0	50.1	50.1	49.0	48.3	246.5	154.5	71.1	28.9
15A	49.0	50.1	50.1	49.0	48.3	246.5	138.6	55.2	22.4
16A	147.3	100.2	102.0	100.0*	96.9	546.4	184.8	101.4	18.6
17A	92.1	15.4	34.7	20.0*	40.1	202.3	104.0	20.6	10.2
18A	222.5	219.1	202.5	208.6	228.9	1081.6	255.3	171.9	15.9
19A							76.2	Check	
20A	141.1	65.5	84.8	69.0	89.4	449.8	200.2	116.8	26.0
1B							79.8		
2B							94.3		
3B							92.9		
4B							92.6	Check	
5B	173.5	169.0	152.3	159.7	180.5	835.0	167.3	72.7	8.70
6B	215.8	221.8	203.5	129.7	147.5	918.3	189.0	94.4	10.28
7B							100.3	5.7	
8B	24.5	25.0	25.0	24.5	24.1	123.1	142.9	48.3	39.2
9B	49.0	50.1	50.1	49.0	48.3	246.5	158.6	64.0	26.0
10B	49.0	50.1	50.1	49.0	48.3	246.5	182.8	88.2	35.8
11B	49.0	50.1	50.1	49.0	48.3	246.5	193.4	98.8	40.1
12B	49.0	50.1	50.1	49.0	48.3	246.5	172.3	77.7	31.5
13B	49.0	50.1	50.1	49.0	48.3	246.5	165.4	70.8	28.7
14B	49.0	50.1	50.1	49.0	48.3	246.5	157.6	63.0	25.6
15B	49.0	50.1	50.1	49.0	48.3	246.5	160.8	66.2	26.9
16B	147.3	100.2	102.0	100.0*	96.9	546.4	181.9	87.3	16.0
17B	92.1	15.4	34.7	20.0*	40.1	202.3	180.6	86.0	42.5
18B	222.5	219.1	202.5	208.6	228.9	1081.6	263.3	168.7	15.6
19B							96.67	Check	
20B	141.1	65.5	84.8	69.0	89.4	449.8	224.1	129.5	28.8

* Estimated; straw and alfalfa hay not analyzed.

TABLE 10

Percentage of total nitrogen and carbon in the unlimed and the limed soils in 1913 and 1917

PLOT NUMBER	FERTILIZER TREATMENT FOR $\frac{1}{10}$ ACRE PLOT	UNLIMED SECTION (A PLOTS)				LIMED SECTION (B PLOTS)			
		Nitrogen		Total carbon		Nitrogen		Total carbon	
		1913	1917	1913	1917	1913	1917	1913	1917
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Nothing	0.1059	0.1033	1.39	1.33	0.0862	0.0770	1.06	0.90
2	16 pounds muriate of potash	0.1098	0.1173	1.44	1.33	0.0871	0.0790	1.14	0.96
3	32 pounds acid phosphate	0.1042	0.1139	1.28	1.24	0.0821	0.0751	1.07	0.86
4	*Minerals only	0.1180	0.1088	1.36	1.26	0.0855	0.0779	1.15	1.04
5	Minerals, 1600 pounds cow manure	0.1002	0.1185	1.38	1.44	0.1030	0.1143	1.44	1.42
6	Minerals, 1600 pounds horse manure	0.0929	0.1230	1.39	1.57	0.1028	0.1190	1.43	1.48
7	Nothing	0.0790	0.0785	0.95	0.93	0.0879	0.0821	1.17	1.02
8	Minerals, 8 pounds $NaNO_3$	0.0880	0.0883	1.11	1.03	0.0801	0.0787	1.08	1.01
9	Minerals, 16 pounds $NaNO_3$	0.0921	0.0975	1.15	1.13	0.0753	0.0810	1.08	0.96
10	Minerals, $Ca(NO_3)_2$ equivalent to 16 pounds $NaNO_3$	0.0941	0.0903	1.14	1.10	0.0805	0.0779	1.12	1.01
11	Minerals, $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$	0.0977	0.0904	1.14	1.21	0.0801	0.0819	1.02	1.06
12	Minerals, $CaCN_2$ equivalent to 16 pounds $NaNO_3$	0.0869	0.0844	1.03	1.02	0.0861	0.0896	1.16	1.07
13	Minerals, dried blood equivalent to 16 pounds $NaNO_3$	0.0925	0.1063	1.14	1.23	0.0807	0.0826	1.05	0.98
14	Minerals, fish equivalent to 16 pounds $NaNO_3$	0.1014	0.1058	1.26	1.22	0.0779	0.0889	1.08	0.93
15	Minerals, concentrated tankage equivalent to 16 pounds $NaNO_3$	0.0884	0.0919	1.21	1.18	0.0717	0.0803	0.93	0.89
16	Minerals, 200 pounds alfalfa hay	0.0891	0.1012	1.20	1.14	0.0897	0.0904	1.18	1.10
17	Minerals, 200 pounds wheat or rye straw	0.0950	0.0980	1.26	1.08	0.0953	0.0952	1.25	1.15
18	Minerals, 1600 pounds cow manure and 16 pounds $NaNO_3$	0.1088	0.1170	1.47	1.36	0.1014	0.1190	1.41	1.48
19	Minerals only	0.0980	0.0872	1.29	1.01	0.0809	0.0784	1.11	0.95
20	Minerals, 200 pounds wheat or rye straw and 16 pounds $NaNO_3$	0.0959	0.0908	1.23	1.03	0.0832	0.0903	1.14	1.12
Average		0.0969	0.1006	1.24	1.19	0.0859	0.0879	1.15	1.07

* Minerals—32 pounds acid phosphate and 16 pounds muriate of potash.

An examination of the average nitrogen content for all the unlimed plots shows that there is now approximately 0.01 per cent, or 200 pounds per plowed acre less nitrogen in these soils than there was in 1909. The corresponding loss from the limed section amounts to nearly 500 pounds of nitrogen per acre. Thus the limed plots have lost about 250 pounds more of

nitrogen per acre than the unlimed plots. The probable reason for this greater loss from the limed than from the unlimed section has been discussed in an earlier paper (2) and only brief reference need be made to it here. It would appear in the case of the unlimed plots that the acidity of the soil has acted as a check on the breaking up of organic matter and therefore on the loss of nitrogen. On the other hand the well limed plots furnished a favorable medium for those organisms that destroy organic matter and as a consequence the loss of nitrogen was heavy. We have here a verification of the popular view that lime tends to "burn out" the organic matter; only the "burning out" must be recognized as an indirect rather than a direct result of the lime. This rather rapid oxidation of organic matter results in the formation of available nitrogen compounds which should be utilized by the growing crops.

In this case, however, it will be remembered that there was very little more dry matter produced on the limed than on the unlimed plots. On these plots nitrogen was *not* the limiting factor and the extra amount of available nitrogen compounds was largely lost. From this we may conclude that on soils that are not especially heavy, and with non-legume crops, oxidation may proceed rapidly enough without the stimulating effect of lime compounds.

It is interesting to note that during the period 1913–1917 there was little change in the average nitrogen content of soil in the two sections. Such change as there was is in the direction of a gain. It is possible that in 1913 the equilibrium of which Russell speaks had been reached and from this time on the gains equaled the losses. If this explanation is accepted it must be admitted that the equilibrium point was lower for the limed than for the unlimed section.

It is of especial interest to note that the nitrogen content of those plots which have received manure and manure with nitrate of soda has been maintained just about on a level with the nitrogen content of the original soil.

The high nitrogen and carbon content of plots 1A to 4A which during the 10 years have received no nitrogen tends to confirm the suggestion which has already been made, viz.: that the soil was naturally better here than in the remaining part of the section. A nitrogen content of 0.079 per cent for 7A illustrates well what will happen when land is cropped continuously without fertilizers or manure. This plot shows a greater loss of nitrogen than any of the others in this section. Assuming that it originally contained 0.1118 per cent, which represents the average of the plots which were sampled in 1909, it has lost nitrogen at the rate of about 600 pounds to the plowed acre.

In most cases, as has already been pointed out, the limed plots have lost more nitrogen than the corresponding unlimed plots, but it happens that this is not true of 7B which is the limed unfertilized plot.

The average percentage of carbon in samples from the unlimed plots is practically the same in 1913 as in the orginal soil, but the limed section shows a decrease during this period of about 0.07 per cent, or 1400 pounds to the

plowed acre. This only emphasizes what has been said with regard to the more rapid oxidation of organic matter on the limed plots.

It will be noted also that the average per cent of carbon is less for both sections in 1917 than it was in 1913. On the other hand, plots 5, 6 and 18 in each section show a decided increase in percentage of carbon over the original soil, the carbon content of which was 1.22 per cent. These plots receive an annual application of manure at the rate of 16 tons per acre and an increase in carbon content is to be expected. The large amount of nitrogen that is supplied causes a rank growth of grain and grass and thus there is left on these plots a heavier stubble or sod than on the plots that receive less nitrogen. These residues supplement the organic matter which is supplied by the manure.

There is quite a contrast between 6A and 7A which lie side by side. In 1917 the former contained 1.57 per cent of carbon and the latter (the no-fertilizer plot) 0.93 per cent. Even on the limed section the nitrogen content of 6A has been increased.

It is of interest to compare the average nitrogen and carbon in the soil from the seven plots (9 to 15) which receive equal amounts of nitrogen with the average for all the plots. The averages are as follows:

TREATMENT	NITROGEN		CARBON	
	1913	1917	1913	1917
	per cent	*per cent*	*per cent*	*per cent*
Plots 9 to 15, unlimed	0.0933	0.0952	1.15	1.16
General average, unlimed	0.0969	0.1006	1.24	1.19
Plots 9 to 15, limed	0.0789	0.0832	1.06	0.99
General average, limed	0.0859	0.0876	1.15	1.07

Here, as in the general average, there is less nitrogen and carbon in the soil of the limed than of the unlimed section. In all cases, too, the average for these seven plots is less than the general average for the twenty plots.

From this it is evident that the heavy applications of manure have aided very materially in bringing up the general average.

SUMMARY

This is a report for the second 5-year period of work that was started in 1908, the object of which is a study of the relative availability of different nitrogenous materials, and of nitrogen losses under a 5-year rotation of corn, oats, wheat and two years of timothy.

In laying out the experiment forty 1/20-acre plots were provided in parallel sections of twenty plots each, so that the nitrogen work could be duplicated, the one section designated as "A" remaining unlimed and the other designated as "B" to be limed at stated intervals.

Both sections have been supplied with liberal amounts of phosphorus and potassium so that these might not become limiting factors.

With slight exceptions the mineral nitrogenous materials have increased the yields over the yields from the check plots.

The average yields of dry matter, and the percentage of nitrogen recovered have been greater with the mineral nitrogenous materials than with organic materials.

Of the four mineral materials, nitrate of soda gave the largest yields of dry matter, and the highest percentage of nitrogen recovered on the unlimed section, and sulfate of ammonia the highest on the limed section.

Of the three commercial organic materials, dried fish gave the highest yields of dry matter and the highest percentage of nitrogen recovered on the unlimed section, and dried blood the highest on the limed section.

Farm manure, and farm manure with nitrate of soda gave the largest total yields, but on account of the large excess of nitrogen supplied by these materials the increases can hardly be considered profitable when compared with the increase made by the commercial nitrogenous materials.

The four mineral nitrogenous materials gave for the period 1913–1917 an average recovery of 29.4 per cent of nitrogen for the unlimed section and 33.3 per cent for the limed section. The corresponding figures for the three organic materials are 23.5 per cent and 27.1 per cent. There is thus shown an average loss of more than two-thirds of the applied nitrogen.

With the exception of the corn crop of 1913 the yields on the limed section were about the same as on the unlimed section.

There is practically no difference in percentage of nitrogen in the dry matter from the limed and the unlimed sections.

Analyses of samples of soil from plots of the two sections show that the limed plots have lost, during the 10 years, nearly 250 pounds more of nitrogen than the unlimed plots. The loss of carbon also has been greater on the limed than on the unlimed section.

It is suggested that in the case of soils of this type and with no legume crops in the rotation to furnish additional organic matter, oxidation may go on rapidly enough without the stimulating effect of lime.

The supply of nitrogen and carbon has been best maintained on those plots which have received the farm manure and the farm manure plus nitrate of soda. The carbon content of these plots has even been increased over the carbon content of the original soil.

The work emphasizes the difficulty of maintaining the nitrogen supply of the soil at a high level under continuous cropping to *non-leguminous* crops, even when *commerical fertilizers* are supplied in generous amounts.

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